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NOTICES

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NOTICE

This is the final volume of the *New Zealand Journal of Science and Technology*, of which the last issue will appear in December 1957.

It will be replaced in 1958 by three journals:

New Zealand Journal of Agricultural Research,
published in alternate months, first issue February 1958.

New Zealand Journal of Geology and Geophysics,
published quarterly, first issue February 1958.

New Zealand Journal of Science,
published quarterly, first issue March 1958.

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EFFECTS OF CHANGES IN LIGHT AND TEMPERATURE ON ROOTS OF PERENNIAL RYEGRASS (*Lolium perenne* L.)

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(*Received for publication, 15 May 1957*)

Summary

Plants of *Lolium perenne* were grown in full spring daylight at a mean temperature of 55°F. at Palmerston North, latitude 40° 30′ S., until the fifth leaf had appeared on the main stem. They were then subjected to various conditions of light intensity and temperature.

A change to lower light intensity, or to higher temperature with a moderate reduction in light intensity, had little effect on the root system compared with a change to higher temperature with a severe reduction in light intensity. Under the latter treatment the cortex of existing roots became brown and shrunken, and sloughed off. The growing points continued to function but formed much thinner roots with fewer and smaller cells in both cortex and stele. New roots were slender and sparingly branched.

A return to the initial conditions resulted in an increase in diameter of the growing parts of existing roots and of new roots.

A combination of lower light intensity and higher temperature reduced both root and shoot weight, but had a greater effect on root weight. By contrast, a change back to full light and lower temperature resulted in a larger increase in root weight than in shoot weight, such that the root-shoot ratio exceeded that for plants grown continuously under these conditions.

These effects are discussed in relation to shoot growth and nutrition.

INTRODUCTION

Roots serve not only for anchorage and for absorption of water and nutrients, but also for the storage of reserves. The last function is closely related to the growth of the above-ground parts of the plant which is influenced by seasonal changes. Comprehensive reviews of literature on relationships between root and top growth are given by Weinmann (1948) and Troughton (1957).

A number of workers report that root growth is usually most active during the cooler months and often ceases during the summer. Darrow

*Now Mrs. C. S. W. Reid.

(1939) found that at lower temperatures the roots of *Poa pratensis* were thick, white, succulent, and coarsely branched, whereas at high temperatures they were small, light brown, and very finely branched. Harrison (1934) found that root and rhizome weights of plants of *P. pratensis* grown at 60°F. exceeded those grown at 80°F. and 100°F. According to Sprague (1944) root weights of several pasture species increased with a rise in temperature up to a certain optimum. The effect of a rise in temperature was, however, to increase root growth proportionally less than top growth so that the root-top ratio, in general, decreased with a rise in temperature. Mitchell (1954, 1955) found similar effects in perennial ryegrass, cocksfoot, and paspalum.

Few investigations have been carried out concerning the effect of light on the development of underground parts of grasses, and most of them deal with photoperiodic responses. Generally, shade reduces the weight, number, and size of underground organs (Harrison, 1934; Watkins, 1940; Mitchell, 1954, 1955; Mitchell and Coles, 1955). Reid (1933) working with *Agrostis canina* and *A. palustris,* and Mitchell (1954) with *L. perenne,* found that root growth was more affected than shoot growth, while Benedict (1941), working with *Agropyron cristatum, A. smithii,* and *Bouteloua gracilis,* reported that the distribution of weight between root and shoot showed no distinct trends.

Jacques and Schwass (1956) have described seasonal changes in the root systems of three grasses. In perennial ryegrass they found that new "white" roots are produced following germination, and again, after a period of little or no activity in summer, from early autumn until late winter, after which the rate declines. Schwass and Jacques (1956) discuss the relationship between the root system and top growth.

The effects of shading at high temperature, as against full daylight at an average New Zealand spring or autumn temperature, on the morphology and anatomy of vegetative plants of *L. perenne* are described elsewhere (Soper and Mitchell, 1956; Mitchell and Soper, in preparation). The present paper describes in greater detail the responses of the root system to changes in one or both factors of full daylight and cool temperature.

MATERIALS AND METHODS

The experiment was carried out at Palmerston North, latitude 40° 30'S., during the months of September to December, 1955. Plants of *L. perenne* were grown in vermiculite supplied with Hoagland's nutrient solution. For morphological and anatomical investigations, plants were grown singly in pots. For dry-weight measurements, six plants were grown per pot and weighed in bulk. Until the fifth leaf had appeared on the main stem (approximately five weeks) all plants were grown out-of-doors in full daylight, during which time the mean temperature was 55°F. The pots were then divided into seven groups, each group comprising six pots of single plants and one pot of six plants. Subsequent treatments and harvesting times are given below.

30% daylight was obtained by shading out-of-doors, 70% daylight by the use of a glasshouse, 20% daylight by shading in the glasshouse and the higher temperature by heating the glasshouse to a temperature which was never less than 75°F. but sometimes considerably more.

	From Appearance of 5th Leaf to Appearance of 9th Leaf (3–5 weeks)		From Appearance of 9th Leaf until 3–5 Weeks Later	
	% Outdoor Daylight	Mean Temp. °F.	% Outdoor Daylight	Mean Temp. °F.
Group 1	100	55*		
„ 2	100	55	100	55*
„ 3	30	55*		
„ 4	70	>75*		
„ 5	20	>75*		
„ 6	20	>75	20	>75*
„ 7	20	>75	100	55*

*harvested

Material for sectioning was fixed in formalin acetic alcohol, a mixture of 5 parts formalin, 5 parts acetic acid, and 90 parts 70% ethyl alcohol. Sections were cut by hand and stained in safranin.

RESULTS

Morphology of the Ryegrass Root System

Fig. 1 shows a typical plant from Group 2 (unshaded, outdoor conditions), approximately 13 weeks old. It had 13 leaves on the main stem, 270 tillers, and 290 roots (Table 1) of a maximum length of 30 cm. About one-third of the roots were thick, white and unbranched, and up to 12 cm. long. The rest were branched, the earlier-formed roots having many secondary branches along the proximal two-thirds of their length, and inconspicuous tertiaries. All the roots had root hairs throughout their length except for $\frac{1}{2}$ cm. behind the tip. The hairs were 1–1$\frac{1}{2}$ mm. long and formed a thick mat about each root.

At the ninth-leaf stage there were no clear-cut differences between either the shoot or the root systems of plants in Groups 1 to 4. Data for Groups 3 and 4 are therefore not presented. In experiments where similar conditions applied, Mitchell (1954, 1955) reported differences from such treatments but in these cases the plants were grown for longer periods under the final treatments.

Plants that had been subjected to considerable changes in both light and temperature (Groups 5 and 6), however, showed large differences from the plants in Groups 1 and 2. Fig. 2 shows a typical plant from Group 6, of the same age as that shown in Fig. 1, with 11 leaves on the main stem, 20 tillers, and 60 roots of a maximum length of 34 cm. It had rapidly acquired features characteristic of plants grown continuously under low light and high temperature (Soper and Mitchell, 1956; Mitchell and Soper, in preparation). Both the shoot and root system were reduced. Leaves that were developing at the time of the change and all those formed after the change were long and narrow. Approximately two-thirds of the roots were fine, the youngest unbranched, the earlier-formed sparingly branched. Root hairs were present over

Fig. 1.—Plant grown in full daylight at a mean temperature of 55°F. (Group 2): approximately 13 weeks old.

Fig. 2.—Plant, of the same age as that shown in Fig. 1, which had been changed when 5 weeks old from full daylight at 55°F. to 20% daylight at high temperature (Group 6).

the surface of the root as in the unshaded, outdoor plant, but were much less densely spaced. The cortex on the proximal (oldest) parts of the remaining roots was either brown and shrunken, or sloughed off, while the distal parts were narrower and the cortex intact. Such a root is shown in Fig. 3, together with a root of comparable age from a Group 2 plant. Fig. 4 shows the region where the change in diameter had occurred. Of the portion shown, the uppermost part consisted of stele only, while below that the cortex was still present as a brown "skin". Sometimes brown epidermis and root hairs also remained. Further down, the root narrowed abruptly and continued entire with narrow cortex and stele.

The result of the reverse change, that of transferring plants from low light and high temperature back to unshaded, outdoor conditions (Group 7), may be seen from Fig. 5 which shows a plant from Group 7, 21 days after its removal to the outdoor conditions. It is of the same age as the plants shown in Figs. 1 and 2. Both top and root system were changing to those characteristic of an unshaded, outdoor plant. There were already 100 tillers, and 136 roots (Table 1), more than twice the number in a plant of comparable age left under low light and high temperature. Of the later-formed roots, some were thicker at the distal than at the proximal end, and some were thick throughout their length. Branching had increased.

Dry-weight ratios of roots to shoot are shown in Table 1. In Groups 1 and 2 and in Groups 5 and 6 the weights of the root systems were approximately proportional to root numbers. Under low light and high temperature (Groups 5 and 6) root weight and number and also shoot weight had doubled in the 3 weeks between the two harvest

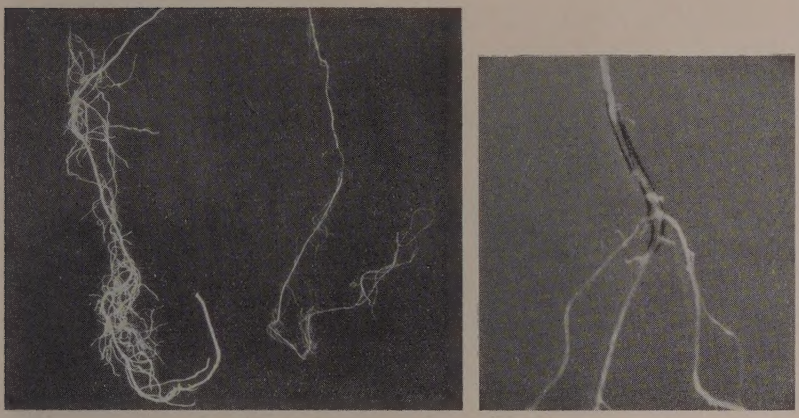


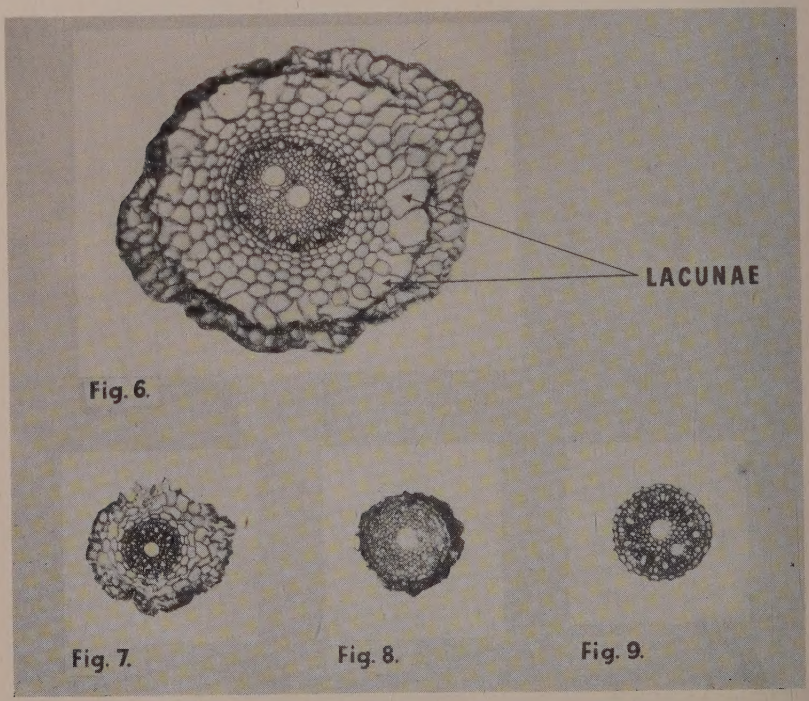
Fig. 3.—Branched roots of comparable age from the plants shown in Fig. 1 (left) and Fig. 2 (right).

Fig. 4.—Portion of the root shown on the right in Fig. 3. The upper part consists of stele only, while below that the brown cortex is still present. The lower part is narrower and the cortex entire. The region where the change in diameter occurs corresponds to the growing region at the time of the transfer from high light and low temperature to low light and high temperature.

Fig. 5.—Plant, of the same age as those shown in Fig. 1 and 2 (13 weeks), which had been changed when 10 weeks old from 20% daylight at high temperature back to full daylight at 55°F.

TABLE 1. NUMBER AND WEIGHT OF ROOTS AND WEIGHT OF SHOOTS AT HARVEST

Group	Length of Total Growth Period (days)	Mean Root No. per Plant	Mean Total Root Wt. per Plant (g.)	Mean Shoot Wt. per Plant (g.)	Ratio Root Wt. to Shoot Wt.
1	56	49	0.42	2.38	0.18
2	91	290	2.27	10.73	0.21
5	70	30	0.05	0.32	0.14
6	91	61	0.10	0.79	0.13
7	91	136	0.96	3.15	0.30



Figs. 6–9.—Transverse sections of roots. Fig. 6. From an unbranched root from an unshaded, outdoor plant (Group 2.). Fig. 7–9 From a plant from shaded, high temperature conditions (Group 6). Fig. 7. From a root of comparable age to that of Fig 6. Fig. 8. From the proximal end of a root which had begun its growth under unshaded, outdoor conditions. The transfer to shaded, high temperature conditions resulted in browning, shrinking and sloughing of the cortex. Fig. 9. As Fig. 8, showing the cortex sloughed off, leaving the endodermis surrounding the stele.
(All × 60).

dates. By contrast, 3 weeks after plants were changed to high light and low temperature (Group 7), root numbers had increased 4 times but total root weight had increased 20 times, while shoot weight had increased 10 times, so that the ratio of total root to shoot weight was doubled and was greater than that for plants grown continuously under unshaded, outdoor conditions (Groups 1 and 2).

Anatomy of the Roots

The changes in morphology recorded above were accompanied by changes in the anatomy of the roots. Fig. 6 shows a transverse section of the proximal part of an unbranched root from an unshaded, outdoor plant (Group 2). Earlier-formed roots were somewhat smaller than this as may be seen by comparing unbranched roots from Groups 1 and

TABLE 2. DIMENSIONS OF CROSS-SECTIONS OF ROOTS
Means and standard errors given for 6 roots

Group	Type of Root	Root Diam. (μ)	Stele Diam. (μ)	Stele/ Root	Cortex Width (μ)	No. of Cells across Width of Cortex	Size of Cells in Cortex (μ)
1	Branched	665 ± 20	228 ± 13	0.34	218 ± 11	8.0 ± 0.0	27 ± 1
1	Unbranched	656 ± 49	280 ± 26	0.43	188 ± 14	9.5 ± 0.5	20 ± 1
2	Unbranched	790 ± 19	283 ± 12	0.36	253 ± 8	9.6 ± 0.2	26 ± 1
5	Branched, with browned cortex at proximal end:—						
	A. proximal:						
	a. browned cortex present*	410 ± 70	196 ± 6	0.48	106 ± 38	7.5 ± 0.5	23 ± 1
	b. cortex sloughed off						
	1. entirely	186 ± 18	186 ± 18	1.00	—	—	—
	2. partly	322	210	0.65	56	—	—
	B. distal: cortex present, unbrowned	298 ± 22	130 ± 18	0.44	84 ± 12	5.7 ± 0.3	15 ± 3
5	Unbranched	395 ± 24	165 ± 9	0.42	115 ± 7	7.0 ± 0.0	16 ± 1
6	Unbranched	389 ± 35	172 ± 17	0.44	108 ± 8	6.7 ± 0.3	16 ± 1
7	Branched, fine at proximal end, thicker at distal:—						
	A. proximal	424 ± 38	158 ± 11	0.37	133 ± 14	6.0 ± 0.0	22 ± 2
	B. distal	658 ± 8	206 ± 5	0.31	227 ± 5	7.7 ± 0.3	29 ± 1
7	Unbranched	641 ± 38	241 ± 17	0.38	200 ± 14	7.8 ± 0.3	27 ± 2

*sometimes shrunken

2 (Table 2). Fig. 7 shows a transverse section of a comparable root from low light and high temperature conditions (Group 6). In the latter root both cortex and stele were narrower than in Groups 1 and 2 although the ratios of stele to root were approximately the same (Table 2). There were fewer and smaller cells in the cortex (Table 2). A feature of the cortex was the absence of lacunae; these were generally present in the outdoor plants (Fig. 6). The number of protoxylem groups in the stele was less, ranging from 7 to 10 as compared with 10 to 12 in the outdoor plants. Similarly with the metaxylem vessels, the numbers ranging from 1 to 2 and 1 to 4 respectively. The diameter of the largest metaxylem vessels was 27 $\pm$ 3 μ in the Group 6 plants and 37 $\pm$ 9 μ in the outdoor plants.

The changes brought about in root structure when plants grown in full daylight out-of-doors were transferred to low light and high temperature are shown in Figs 7–9. Figs. 8 and 9 are sections from the proximal end of a root that had begun its growth under outdoor conditions. Fig. 8 is from a part of the root where the cortex was brown and shrunken and in Fig. 9 the cortex had sloughed off leaving the endodermis surrounding the stele. The dimensions of the stele were comparable with those from the earliest-formed roots of outdoor plants (Table 2). The distal part of the root was slightly narrower than a root that had undergone its entire development after the change to low light and high temperature (Table 2). Later-formed roots were fine and unbranched or sparingly branched. In some of these the cortex was sloughing off although it was not discoloured. It would appear that the cortex of roots grown for any length of time under conditions of low light and high temperature has a relatively short life.

The reverse change, that from shaded glasshouse back to unshaded outdoor conditions (Group 7), resulted in an increase in thickness of subsequently formed roots and distal parts of fine roots (Table 2). These thicker parts had some lacunae and a greater number of larger cells in the cortex (Table 2) and more protoxylem groups and larger metaxylem vessels in the stele.

Discussion

Under conditions of full spring daylight and cool temperatures, shoot and root growth were vigorous. At first each nodal root was unbranched but, as growth continued, numerous branches developed. All roots were thickly covered, except at the tip, with root hairs, so that the surface area for absorption was many times greater than that of the roots themselves.

By contrast, shading and an increase in temperature had an adverse effect on both shoot and root growth. Both root and shoot weights were reduced. The effect on root weights was however greater than on shoot weight so that the root-shoot ratio was reduced. The decrease in root weight was due to the shrinking and sloughing of the cortex from the roots and also to the fact that, although the growing point of the root continued to function, it formed a much narrower root. It may be inferred that, whereas the original conditions favoured the production in the shoot of carbohydrates in quantities ample for the promotion of vigorous new root growth, the combination of shading and high temperatures, by reducing the rate of formation of photosynthetic products and increasing the loss by respiration, resulted in a drastic

reduction in the amount available for root growth. This may have been further intensified by decreased translocation of sugars from the leaves at the higher temperature, as in the tomato (Went and Hull, 1949).

With conditions changed to reduce photosynthesis and to increase respiration, there would have been a large drain on the stored reserves throughout the plant. It seems likely that the withering up of the cortical cells occurred when the reserves were withdrawn from the root cortex, as suggested elsewhere by Jacques (1956). Any carbohydrates produced subsequently were sufficient to form only a narrower root. That this narrower root was the result of a decrease in both cell division in the growing region and in cell size is shown by the smaller number and size of cells in cross-sections of the mature root (Fig. 7). Cortex and stele, although smaller, were produced in the same proportions as before. The stele in the upper part of the root remained intact, thus forming a conducting channel between the new absorbing region and the base of the shoot. New roots continued to be formed under these conditions although on a reduced scale and were fine and sparingly branched. The absorptive and storage capacities of the root system as a whole would be much less than that of a plant grown in full daylight at the cooler temperature.

The change back to the original, more favourable, conditions had the reverse effect. Growth of both roots and shoot increased. Root weights, however, increased proportionally faster than shoot weights. A probable explanation is as follows. Shading at high temperatures retards root growth and the development of new tillers, the inhibitory effect on the tillers being the greater (Soper and Mitchell, 1956). A change to full daylight results in the formation of increasingly larger amounts of photosynthetic products in the leaves while lower temperatures reduce respiration. Supplies available for shoot growth and for translocation to the roots are therefore increased. Whereas food can go directly to the growing points of existing roots and be used in increasing their diameter and absorptive capacity as well as in the development of new roots, only some of the inhibited tiller buds respond to increased food supplies (Mitchell, 1953; Soper and Mitchell, 1956), although new tiller buds develop unchecked. The absorption of more water and nutrients by the increasingly efficient root system, together with the improvement in carbohydrate supplies as the new tillers and successive tiller buds develop, will gradually restore the original root-shoot ratio.

ACKNOWLEDGEMENT

The writer is indebted to Mr. H. Drake for assistance with the photography.

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GOITROGENIC EFFECT OF WHITE CLOVER (*Trifolium repens* L.). II.

By G. W. Butler[1], D. S. Flux[2], G. B. Petersen[3], E. W. Wright[4], A. C. Glenday[1], and J. M. Johnson[1]

(Received for Publication, 1 May 1957)

Summary

Two groups of sheep were grazed on cyanogenetic and non-cyanogenetic white clover for 43 days. At the end of this period no significant differences between groups were observed in the incorporation of ^{131}I into the thyroids or in the heights of the acinar cells. The sheep fed on cyanogenetic clover showed a decrease in the total iodine content of the thyroid per unit fresh weight.

In a *in vitro* experiment it was shown that the levels of thiocyanate occurring in the blood sera of sheep grazing CN clover inhibited the conversion of inorganic iodide to organically bound iodine by freshly excised thyroid slices.

The effect of periodic injections of thiocyanate and the time elapsing between the last injection of thiocyanate and intraperitoneal injection of ^{131}I iodide on the rate of incorporation of ^{131}I into the thyroid was examined in rats. Both factors were found to influence the rate of ^{131}I incorporation into the thyroids.

Introduction

In a previous communication (Flux *et al.*, 1956) it was reported that the herbage of cyanogenetic white clover* exerted a goitrogenic effect when fed to guinea-pigs and rats. An increase in the thiocyanate level of the sera of the test animals was also observed, and it appeared that the goitrogenic action arose from this cause. It was also shown that the sera of sheep fed CN white clover contained concentrations of thiocyanate of the same order as those which had been associated with the goitrogenic action in guinea-pigs and rats.

In this paper the first experiment described was designed to test the possibility of a goitrogenic effect of CN clover when fed to sheep. Secondly, the sensitivity of inhibition by thiocyanate ion of the incorporation of ^{131}I-iodide into slices from freshly excised thyroid glands of sheep is reported. Finally, an experiment with rats to examine further the nature of the interaction between thiocyanate ion and the thyroid gland is presented.

Sheep-feeding Experiment

Method

Ten Romney-Southdown crossbred wethers of age 2 to 3 years, which had grazed continuously on pasture on a low-iodine soil, were selected for the experiment and divided randomly into 2 groups. Pure

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[3]Present address: Department of Biochemistry, University of Oxford, England.

[4]Biochemist, Animal Research Station, Wallaceville.

*Cyanogenetic and non-cyanogenetic white clover will be referred to as CN clover and non-CN clover respectively.

stands of CN and non-CN clover were sown; the area was divided into three blocks and each block was divided into two paddocks randomly allotted to the two treatments. The 2 groups of animals were rotationally grazed round the blocks during the feeding period of 43 days, one group remaining on each of the types of clover throughout. On the 43rd day, the animals were slaughtered and the thyroids removed for histological and chemical examination. A portion of each lobe was fixed in 5% acetic acid Zenker's solution and another portion was stored in the deep-frozen state pending analysis.

The following measurements were made during the experiment. Unless otherwise stated, the methods used have been described or cited in an earlier communication (Flux *et al.*, 1956).

Picric acid test for degree of contamination of non-CN white clover stands with CN clover.

Serum thiocyanate analyses of the bloods of the test animals at intervals during the feeding period.

Thyroid thiocyanate analyses at the end of the feeding trial. The tissue was freeze-dried and ground and 1-g. aliquots were homogenized in water, made to 50 ml., deproteinized and concentrated on a water bath to 10 ml.; 5-ml. aliquots were taken for SCN estimation.

Mean heights of the thyroid follicular cells in each animal were measured.

Total thyroid iodine analyses at the conclusion of the feeding period for each animal. For each determination, several slices were taken randomly from portions of each lobe, to give a sample of ca. 0.6 g. fresh weight.

Measurement of the ^{131}I incorporation into the thyroids in vivo. On the 33rd day the animals were injected intraperitoneally with 60 μC. of carrier-free ^{131}I-iodide and the time course of the incorporation of ^{131}I into the thyroids was determined over a period of 10 days. During measurements, the sheep were held in a cradle harness which ensured that the thyroids were situated in a reproducible position for each measurement. A PbG10 lead-cathode detecting tube was situated about 22 cm. from the thyroid, suitably screened from general radiation from the animal. The technique of measurement will be described in detail elsewhere (Wright, unpublished).

Results

The picric acid test showed that the non-CN stand was not contaminated with CN clover.

TABLE 1. THIOCYANATE CONTENT OF SERA OF SHEEP GRAZING ON CN AND NON-CN CLOVER

	No. of Animals	SCN (mg%) on 19th Day	31st Day	43rd Day
Non-CN clover	5	2.1 ± 0.2	2.0 ± 0.01	2.5 ± 0.1
CN clover	5	11.6 ± 0.2	9.9 ± 0.05	9.5 ± 0.2

Serum thiocyanate analyses of the bloods are summarized in Table 1. It will be seen that clear-cut differences were maintained during the feeding period, with little within-group variation.

The thyroid thiocyanate content proved to be too low for accurate measurement and determinations showed only that it was less than 10 mg.% dry weight.

Mean cell heights of the thyroid follicular cells per animal and per group are given in Table 2. It will be seen that there was no significant difference between the mean cell heights in animals fed CN and non-CN clover. There were highly significant differences between animals within both groups. The difference between cells within follicles was the major source of variance, as in the case of similar measurements made on guinea-pigs.

TABLE 2. THYROID DATA FROM SHEEP-FEEDING EXPERIMENT

Animals	Total Iodine μg./g. Wet Tissue	Acinar Cell Heights (μ)
Non-CN group		
Sheep 1	146.8	6.19
2	153.0	5.03
3	144.5	4.53
4	165.5	4.84
5	147.5	5.09
Mean	151.5 ± 3.8	5.14 ± 0.28
CN group		
Sheep 1	133.6	5.91
2	130.0	5.48
3	129.1	5.64
4	128.0	4.48
5	147.8	4.66
Mean	133.7 ± 3.8	5.23 ± 0.28

Total iodine contents per unit fresh weight of thyroid tissue are presented in Table 2. Thyroids from animals fed CN clover had a significantly lower iodine content than those from animals fed non-CN clover.

The ^{131}I activity in the thyroids of sheep grazing on the two strains of white clover at different times after intraperitoneal injection of ^{131}I-iodine is shown in Table 3. In no case did the treatment differences reach significance level. Linear regressions were fitted for each animal between 48 and 216 hr., assuming observation times to be equally spaced at 24-hr. intervals. For no animal did the regression coefficient reach significance level. The test for treatment differences of regression coefficients showed that these were significantly different.

Linear regression coefficients were fitted to the data for each animal between 0 and 24 hours, assuming observation times to be equally spaced at 4-hr. intervals. No significant differences in regression coefficients were observed between groups. All animals showed highly significant regression coefficients in the group fed CN clover, but, of the group fed

TABLE 3. ^{131}I UPTAKE IN THYROIDS OF SHEEP GRAZING CN AND NON-CN CLOVER

Treatment means and standard errors at various times

	4 hr.	8 hr.	16 hr.	24 hr.	32hr.	40 hr.	48 hr.
Fed CN clover	5.794	9.72	11.87	16.74	18.29	19.57	19.10
Fed Non-CN clover	7.222	11.08	11.60	19.04	18.78	17.49	20.72
S.E. (groups)	± 0.567	±1.19	±1.27	±2.59	±2.79	±2.01	±2.30

	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.	192 hr.	216 hr.
Fed CN clover	18.36	18.91	15.71	17.41	16.25	17.35	17.61
Fed Non-CN clover	18.70	17.63	16.18	14.54	17.87	16.51	19.58
S.E. (groups)	±2.06	±2.52	±1.85	±2.02	±2.57	±2.27	±2.31

non-CN clover, only in the case of two animals did the coefficients reach the 5% significance level.

The fresh weights of the thyroid glands varied from 4.8 to 12.2 g. The variation bore no relation to the treatments and was ascribed to previous history of the animals.

INHIBITION BY THIOCYANATE ION OF THE INCORPORATION OF ^{131}I IODIDE INTO ORGANICALLY BOUND IODINE OF SHEEP THYROID SLICES

Using cell-free preparations of thyroid tissue, Taurog *et al.* (1955) showed the conversion of inorganic ^{131}I to organic ^{131}I was completely inhibited by 10^{-3}M thiocyanate. For the present study it was desirable to investigate further the sensitivity of sheep thyroid tissue to inhibition, in order to gauge the effects of various levels of blood SCN. The incorporation of ^{131}I iodide into slices of freshly excised thyroid tissue was therefore studied, using modifications of the technique of Taurog *et al.* For completeness, all the steps are detailed below.

Method

Six whole thyroids were obtained from freshly slaughtered sheep and placed immediately in ice-cold Krebs-Ringer bicarbonate solution, which had previously been gassed with $95\%O_2/5\%CO_2$. Twelve solutions were prepared, each of 3.0 ml., normal with respect of Krebs-Ringer bicarbonate solution and containing in addition 25 μC. ^{131}I in 0.2 g. ^{127}I and the following concentrations of thiocyanate:

Flask No.	SCN concn (mg%)
1–3	0
4–6	4.9
7–9	9.8
10–12	14.7

The solutions were contained in stoppered 25 ml. Erlenmeyer flasks in a water bath maintained at 37°C.

Thyroid slices were prepared by hand and washed with ice-cold Krebs-Ringer bicarbonate solution, then 430–490 mg. fresh weight of slices were placed in each of the flasks and incubated for eight hours with constant shaking. Slices from only one lobe were used for each flask, so that animal variability was present in the experiment. The flasks were then placed in an ice-bath and the incorporation of iodine stopped by addition of 25 μl. of 0.08 M thiouracil prepared as the sodium salt.

One flask from each treatment was then treated as follows: one ml. of the supernatant was removed and placed in a micro-beaker. This was counted and the count obtained compared with the activity of a similar sample from the ^{131}I iodide mixture prepared at the time of the incubation. This gave a measure of the ^{131}I uptake from the solution.

Two flasks from each treatment were treated as follows: Separate homogenates of the contents of these flasks were prepared using a Teflon homogenizer. The pH of the homogenates so obtained was adjusted to 8.0–8.5 with dilute sodium hydroxide solution and a further 0.5 ml. aliquot of each homogenate was hydrolysed with 5–10 mg. of pancreatin at 37.5°C. for eight hours under toluene.

At the end of hydrolysis, 25-μl. aliquots of each product were chromatographed on a 2.5 cm. band on filter paper, in a collidine-water-NH_3 system, as previously described, (Taurog, Tong, and Chaikoff, 1950). As a reference standard to assist in the location of the organic iodine bands, 10 μl. of the radio-iodide standard was placed on a point 2 cm. from the edge of the paper and 2.5 cm. from the end of the band. Radioautographs of the dried papers were prepared to locate the ^{131}I using no-screen X-ray film. The papers were then cut to isolate the bands of protein-bound iodine, amino-acid-bound iodine, and inorganic iodide. The activity of the papers was measured by wrapping them around a PbG10 lead-cathode tube and counting them in the dark (to reduce background).

Results

In Fig. 1, two contrasting radioautographs are shown to illustrate the degree of inhibition of incorporation of iodide into organically-bound iodine, observed in the presence of thiocyanate.

The detailed results of the experiment are given in Table 4, where it will be seen that a marked inhibition of ^{131}I incorporation accompanied increasing concentrations of thiocyanate. There was considerable animal variability observable between flasks.

THIOCYANATE INJECTION EXPERIMENT

In a previous paper (Flux *et al.* 1956) it was postulated that if thiocyanate were administered to animals for a prolonged period, the goitrous thyroids so produced might be expected to have a more or less normal overall iodine exchange. Removal of the goitrogen would then result in enhanced ^{131}I incorporation. This hypothesis was tested as follows:

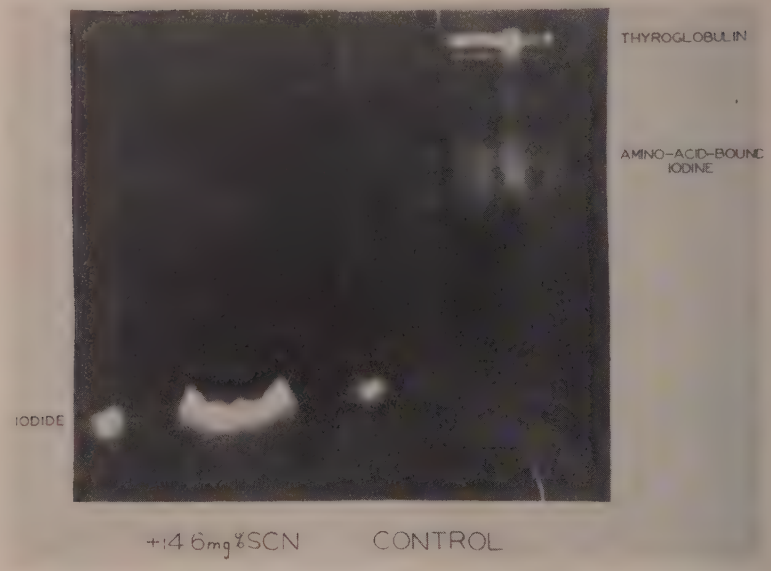

Fig. 1.—Radioautographs showing the inhibition by thiocyanate of the conversion of iodide into organically bound iodine.

TABLE 4. EFFECT OF THIOCYANATE ON THE INCORPORATION OF RADIO-IODIDE INTO THE ORGANIC IODIDE FRACTION OF SLICES FROM EXCISED SHEEP THYROIDS

Flask Number	Treatment	Protein-bound Iodine (c/m)	Amino-acid-bound Iodine (c/m)	Iodine (c/m)	^{131}I Absorbed (%)
1	Control	68	260	133	
2		349	824	346	
3					36
4	+4.9 mg% SCN	80	161	298	
5		83	207	697	
6					10
7	+9.8 mg% SCN	12	24	855	
8		6	47	404	
9					15
10	+14.7 mg% SCN	3	31	431	
11		19	45	468	
12					15

Method

The sixteen freshly weaned female rats used were from the colony kept at the Animal Research Station, Wallaceville, these being derived from the Wistar strain. They were spayed to eliminate the possibility of interference from endogenous oestrogen (Noach 1955) and were maintained on a pelleted mixture of grain meal and dried skim-milk powder to which a vitamin A supplement was added. They were used

for the experiment when six weeks old and were allotted at random to treatments as follows:

Rats 1–4 received a first sub-cutaneous injection of 1 ml. KSCN solution (containing 14.6 mg. SCN per ml.) followed by further injections of 0.5 ml. of the same solution every 8.2 hours for a period of eight days, the injections being stopped 27 hours before injection of ^{131}I iodide.

Rats 5–8 were treated as above, except that the injections were continued until 3 hr. before the time of the ^{131}I injection.

Rats 9–11 received a single injection of 1 ml. thiocyanate solution (i.e. 14.6 mg. SCN) at the same time as rats 5–8 received their *last* injection (i.e., three hours before intraperitoneal injection with ^{131}I iodide).

Rats 12–16 were controls and received no treatment.

All animals were injected intraperitoneally with a dose of 20 μC. ^{131}I iodide contained in 0.15 μg. I as carrier. The counting technique was the same as has been described previously, (Flux *et al.* 1956), and six determinations of the radioactivity in the thyroids of the lightly anaesthetized animals were made at intervals over a period of 300 minutes after the injection of the radio-iodide. Finally, the animals were killed and the thyroids dissected out for determination of radioactivity and total thyroid iodine, and for histological examination. Whole blood was collected for determination of the final level of thiocyanate, the bloods from each treatment being pooled for a single analysis.

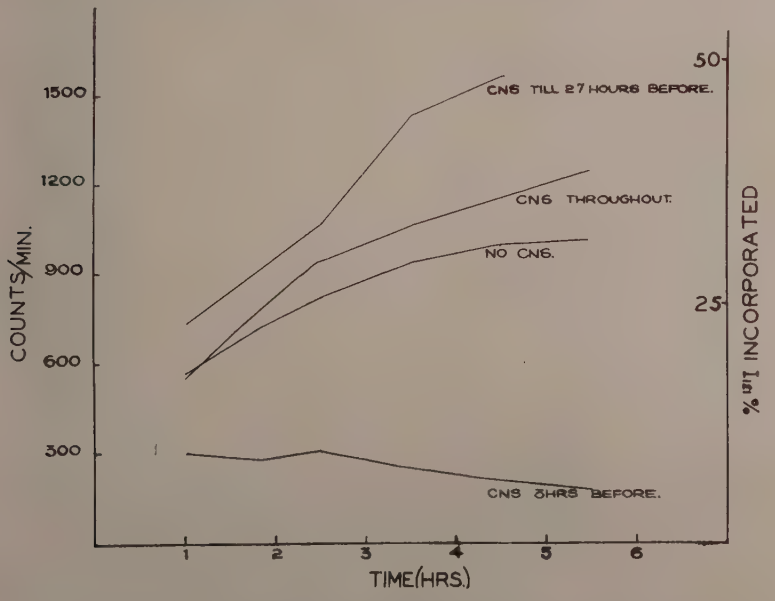

Fig. 2.—Levels of ^{131}I activity of the thyroids of rats at various times after ^{131}I injection, as affected by injections of SCN subcutaneously.

Results

The ^{131}I data are presented in Fig. 2 and Table 5. As counts were made at the same time intervals for all animals, the rate of change was assumed to be constant over any time interval between counts, and the data for the analysis were obtained by interpolation. The times taken were as close to the average times of inspection of the thyroids as possible.

TABLE 5. Mean Thyroid Counts, Standard Errors, and Significant Test Results for the Thiocyanate Injection Experiment

Time (min.)	Treatments				F(3, 11 d.f)
	I CNS till 27 hr. before	II CNS throughout	III CNS 3 hr. before	IV Control	
60	738 ± 56	560 ± 56	304 ± 65	567 ± 56	8.53**
110	927 ± 79	779 ± 79	285 ± 91	723 ± 79	10.07**
150	1064 ± 97	938 ± 97	312 ± 111	828 ± 97	9.55**
210	1426 ± 127	1065 ± 127	252 ± 147	938 ± 127	12.41**
270	1554 ± 111	1155 ± 111	207 ± 128	993 ± 111	21.80**
330	1604 ± 153	1245 ± 153	185 ± 176	1008 ± 153	12.96**

It will be seen from Table 5 that:

(1) Treatment with thiocyanate three hours prior to ^{131}I injection depressed the incorporation of ^{131}I iodide into the thyroid to levels which were significantly lower than all other treatments at all times.

(2) Treatment with thiocyanate at frequent intervals until 27 hours prior to ^{131}I injection resulted in an ^{131}I iodide incorporation which was significantly greater than that for the controls from time 210 minutes onwards.

(3) The ^{131}I incorporation to the thyroids of animals treated with thiocyanate throughout did not differ significantly from that for the controls at any set time.

TABLE 6. Final Levels of Total Iodine and ^{131}I in the Thyroids, and of Thiocyanate in the Sera in the Thiocyanate Injection Experiment

Treatment	^{131}I per Gland (c/m)	Total Thyroid I (μg.)	Serum Thiocyanate (mg.%)
SCN till 27 hr. before	2890 ± 240	0.90 ± 0.17	2.0
SCN throughout	2130 ± 240	0.82 ± 0.17	3.5
SCN 3 hr. before	144 ± 25	1.39 ± 0.20	14.3
Control	1830 ± 240	1.26 ± 0.17	3.3

In Table 6, the data for the final levels of total iodine and ^{131}I in the thyroids are given, together with the final levels of thiocyanate in the sera. With reference to the total thyroid iodines, a comparison of long SCN treatment versus little or no SCN (i.e., treatment (1) and (2) v. (3) and (4)), gave a significant F (7.18 for 1, 11 d.f.), the difference between individual treatments required for significance being 0.54. The SCN concentration of sera from Treatment 2 was lower than expected, but the sample was taken eight hours after injection of 7.3 mg. SCN.

DISCUSSION

In the previous paper on this subject (Flux *et al.*, 1956) it was suggested that the reason for the higher rates of ^{131}I incorporated in rats and guinea pigs which had been fed CN clover could have been a time interval between ingestion of SCN and ^{131}I administration of sufficiently long duration for the thiocyanate inhibition to have disappeared. The thiocyanate injection experiment reported here supports this view. It appears that the type of ^{131}I uptake curve obtained depends on the duration of prior administration of the CN or SCN and also upon the time between the last intake or production within the body of SCN and ^{131}I uptake measurements.

In the experiment where sheep were fed CN clover, despite increased levels of thiocyanate in the blood the only evidence of an effect on the thyroid was a decrease in total iodine content per unit fresh weight. It is likely that the feeding period (43 days) was too short to produce much change in sheep. Another explanation has, however, been suggested by a very recent report of Kennedy* who found that, when the iodine intake of rats was restricted, the ratio of tri-iodothyronine to thyroxine was increased. As tri-iodothyronine is the more active compound in most tests this change might enable animals to accommodate themselves to reduced iodine intake without increased release of "thyroid stimulating hormone" from the anterior pituitary, with consequent changes in the thyroid. Since the animals were fed clover up to and during the measurement of incorporation of ^{131}I iodide into the thyroids, it was to be expected (from the thiocyanate injection experiment) that no significant differences between treatments would be obtained in the ^{131}I measurements.

The *in vitro* experiment showed that concentrations of SCN in the incubation medium, equivalent to those in the blood serum of the sheep in the feeding experiment, inhibited uptake of ^{131}I by the sheep thyroid slices. An important point with regard to this experiment was that the ratios of thiocyanate to inorganic iodide in the media (ranging from 200 to 600) were of the same order as those to be expected in the blood plasma of sheep grazing CN clover, (Taurog *et al.*, 1950, 1955).

In the sheep-feeding experiment, great variability in ^{131}I uptake among sheep in the same treatment group was seen. This was also seen between slices from thyroids of different sheep in the *in vitro* experiment.

It should be pointed out that the work described here and in the preceding paper (Flux *et al.*, 1956) could have relevance with respect to other cyanogenetic forage plants such as certain species of *Lotus*,

*Dietary iodine and Thyroid Function. Communicated to Australian and New Zealand Association for the Advancement of Science, January 1957.

Cynodon, and *Sorghum*, (Finnemore and Cooper, 1938; van der Walt and Steyn, 1941). In any particular case, the iodine content of the herbage and the extent to which thiocyanate levels in the blood of the grazing animal were increased would need to be taken into consideration.

In conclusion, further work will be necessary to establish whether herbage containing cyano-glucosides has a significant goitrogenic action on ruminants, with particular reference to the effect of ingestion of the feed for longer periods than that of 43 days used in the experiment described above.

Acknowledgements

It is with pleasure that the authors acknowledge the assistance of several persons in this work: Professor W. Riddett for provision of land on the Dairy Farm, Massey Agricultural College, for the sheep-feeding experiment; Messrs. L. Corkill, W. G. Thurston, and D. B. Edmond for assistance in the establishment and maintenance of the clover swards; Mr. C. S. W. Reid for assistance in the sheep-feeding experiment; and Messrs. W. Bennett, K. Craven, R. Munford, and W. Smith for technical assistance. We are grateful to Messrs. May and Baker (N.Z.) Ltd. for the gift of a quantity of MCPB weed-killer.

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THE RELATIONSHIP BETWEEN LIVE-WEIGHT AND CARCASS-WEIGHT INCREMENTS OF PIGS

By D. M. Smith, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 24 April 1957*)

Summary

The live-weight to carcass-weight relationship of 425 pigs kept under standard feeding and management conditions and slaughtered between 29 and 240 lb. live weight is presented.

The regression of carcass weight (Y) on live weight (x) was found to be linear, $Y = 0.84x - 4.6 \pm 3.3$ when the regression was based on hot carcass weight and $Y = 0.79x - 4.4 \pm 3.1$ when based on frozen carcass weight (94% of hot carcass weight).

Each pound of live-weight increment yielded 0.84 lb. of hot carcass weight and 0.79 lb. frozen carcass weight at all stages of growth.

The dressing percentage (frozen carcass weight as a percentage of live weight) was found to rise from 64% for pigs of 30 lb. to 77% for pigs of 240 lb. live weight.

Introduction

There is very little information in scientific literature upon the relationship between live and carcass weight of domestic animals from birth or weaning to market weight. This is perhaps not surprising since each species is slaughtered within one or more fairly restricted weight classes, dictated by conventional market requirements.

However, any study of the economics of meat production at various stages of growth requires a knowledge of the relationship between the weight increments measured on the live animal and the marketable meat that these gains represent.

In this paper, the results of a study of the relationship between live weight and carcass weight of pigs fed and managed under standard conditions and slaughtered at various stages between weaning and bacon weight are presented.

Experimental

The live- and carcass-weight records of 425 pigs of the Berkshire and Large White breeds between 29 and 240 lb. were used in this study. Included in this number are 213 records previously reported upon (Smith, 1952). All pigs were fed a standard ration of separated milk or milk and meal according to live weight and a standard pre-weighing and pre-slaughter procedure was followed. All pigs were weighed twice each week from weaning at eight weeks of age until marketed. The last feed was given at 4.30 p.m. on the day prior to weighing. At 5 a.m. the next morning each pen of pigs was roused and driven out into the dunging race and left there to dung and urinate for approximately five minutes. The pigs were then weighed and any pig reaching slaughter weight was drafted from its mates and held without feed until slaughtered between the hours of 10 a.m. and noon of that day.

The animals were processed either at the Station or at a single commercial slaughterhouse. In both cases the carcasses were weighed immediately after dressing—while hot—and the weights recorded were

94% of this hot weight, since it is the practice in slaughterhouses holding export licences in this country to allow for a 6% shrinkage to cover losses in subsequent cooling and freezing.

It is realized that both scientific and commercial practices differ as between institutions and countries in the method of arriving at final dead weight. In some cases, hot dressed weight is used; in others, the weight after cooling for a given period of time; and in others again, an arbitary percentage deduction from hot weight is made. The present figures, as mentioned above, are based on hot weight less 6% but wherever values estimated on this basis have been used they are followed in parenthesis by the actual hot carcass weight.

The method of dressing was as follows:

Pigs below 120 lb. carcass weight were bled, scalded to remove the hair, eviscerated, and weighed. The head, trotters, leaf lard, and kidneys remained on the carcass. Pigs over 120 lb. carcass weight were singed after scalding, but, apart from this one additional process, were weighed in the same condition as those below 120 lb. In every case when pigs were slaughtered, a member of the Station staff recorded the earmark or tattoo and weight of each pig as weighed so that the carcass weight of each individual could be related to its live weight.

Results

The relationship of live weight to frozen-carcass weight for the 425 pigs included in this study is shown in Fig. 1.

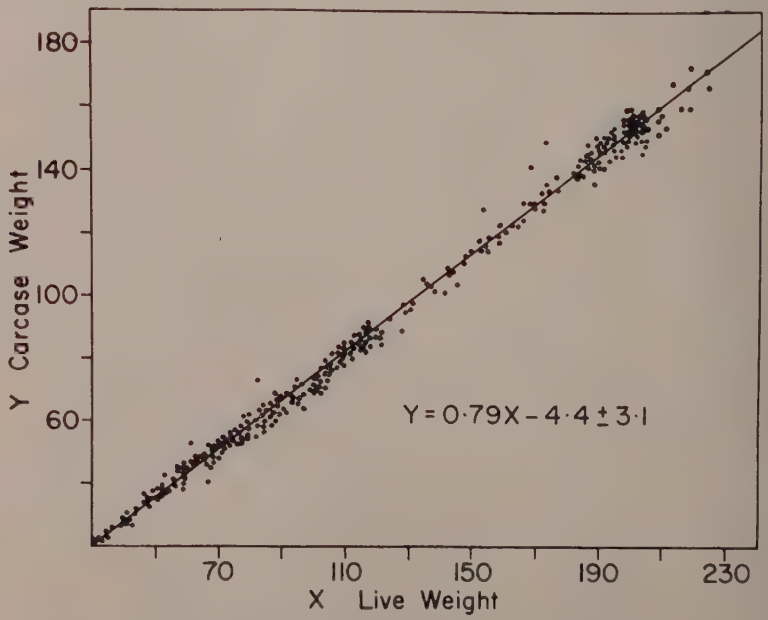

Fig. 1.—The relationship of live weight to frozen-carcass weight.

The regression equation derived from these data was $Y = 0.79x - 4.4 \pm 3.1$ where Y = carcass weight and x = liveweight (1), or $Y = 0.84\,x - 4.7 \pm 3.3$ (2).

Progressive live weights at 10 lb. intervals between 30 and 240 lb. were listed and the corresponding carcass weights calculated using the above regression (1). From these paired values dressing percentages were derived (dressing percentage = carcass weight as a percentage of live weight). The relationship between live weight and dressing percentage is shown in Fig. 2, which illustrates a fact that has long been recognized, that dressing percentage increases with live weight.

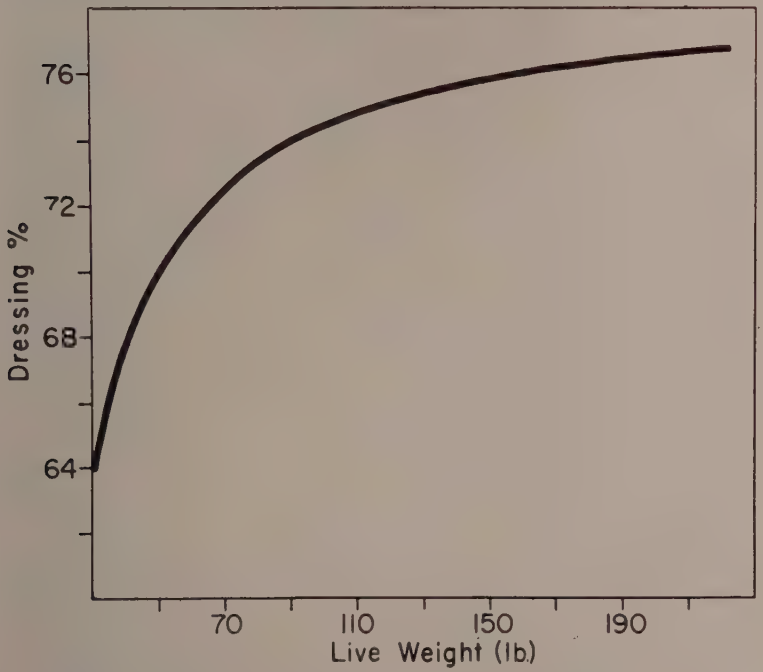

Fig. 2.—The relationship between live weight and dressing percentage.

The values shown here are higher than those published by Menzies-Kitchin (1942) but this difference could be due to the difference in the nature of the feeds used—separated milk as against meal—or to the interval between the last feed and weighing. On the other hand, the values in Fig. 2 differ markedly from those used by Atkinson and Klein (1945, 1946), being lower at lighter weights and greater at the higher weights. To some extent this can be accounted for by differences in method of dressing, but such differences would be expected to be constant throughout the weight range covered.

DISCUSSION

The fact that the dressing percentage of pigs rises with increasing liveweight has been cited by several workers, Davidson (1948), Atkinson

and Klein (1945 and 1946) and Mercer (1928) as favouring the marketing of heavy rather than light animals. These writers imply and Mercer states that the higher dressing percentage of heavy pigs offsets their greater feed requirement per pound of gain. The present author used this premise in a previous paper (Smith, 1952).

As shown in Fig. 1, however, the regression of carcass weight on live weight is linear. Each pound of live-weight gain yields 0.79 (0.84) lb. of carcass irrespective of the absolute liveweight. It follows, therefore, that the extra feed required to produce a pound of gain on a 200 lb. pig as compared with a 100 lb. animal is due to the increased maintenance costs and to the higher energy content of the tissue being laid down by the heavier pig. It also follows that no part of this extra feed requirement per unit of weight gain is offset by higher dressing percentage. The apparent confusion has arisen through applying dressing percentage values to growth increments and assuming that carcass gain per unit of live-weight gain could be derived from the dressing percentage.

That dressing percentage rises with live weight is a consequence of the interplay of two factors:

(1) The linear regression of carcass weight on live weight;

(2) The fact that this regression does not pass through the point of origin: $Y = bx - c$ when Y = carcass weight and x = live weight.

Dressing percentage, which is the ratio of live weight to carcass weight, $= \frac{Y}{x} = b - \frac{c}{x}$. Since both b and c are constants, the value of $\frac{Y}{x}$ must increase as x increases but at a declining rate. Thus, while unit live-weight increments yield constant carcass increments, the dressing percentage rises as the animal grows.

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A NOTE ON THE OCCURRENCE OF THE EUCALYPTUS TORTOISE BEETLE (*Paropsis dilatata* Er.) IN THE NORTH ISLAND

By L. Gurr, Massey Agricultural College, Palmerston North

(*Received for publication, 15 April 1957*)

Clark (1930) in his account of the eucalyptus tortoise beetle (*Paropsis dilatata* Er.) in New Zealand mentions a record of this species from Bulls in 1925, and Dr. David Miller (pers. comm.) confirms this record. Clark however was unable subsequently to find *Paropsis* in the North Island and considered that the beetle was not established in this island; he restated this opinion in a later paper (Clark 1938). Since no further occurrences were reported from the North Island, Clark's opinion was taken as correct and repeated in general works on New Zealand entomology, e.g. Miller (1944) and Cottier (1956).

Between 7 February and 12 March 1956, four different specimens were sent to the author from widely separated localities in and about Palmerston North, while Mr. A. E. Reeves of Messrs. Ivon Watkins Ltd. reported that larvae had caused damage to nursery stock of eucalypts at Bulls during the summer of 1956, and the State Forest Service reported damage to older eucalypt trees and an adult beetle from the same area. This year (1957) damage to eucalypts has occurred throughout the Manawatu and reports of damage have been frequent from all parts of the district, while Mr. Ian Townsend, of the Entomology Division, Department of Scientific and Industrial Research, has found it in the Hawera district, Taranaki, this autumn.

Whether the present outbreak has been caused by the progeny of individuals recently arrived in the district or is merely an eruption of a small struggling population, of which the Bulls specimen, referred to by Clark (1930) was one, it is impossible to say. The former explanation of the origin of the present outbreak seems to be the more likely one, as it is unlikely that even if comparatively rare, such a striking beetle, with a food plant as ubiquitous as *Eucalyptus*, should escape notice for as long as 30 years.

It is clear however that the pest is now well established in the North Island, is spreadingly rapidly, and this year has caused considerable damage to young growth of eucalypts.

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THE ECONOMICS OF USING MEAL SUPPLEMENTS TO A SEPARATED-MILK RATION FOR FATTENING PIGS IN NEW ZEALAND

By D. M. Smith, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 24 April 1957*)

Summary

A graphical method of determining the weight to which pigs can be fed profitably on meal for various meal and meat price ratios is described.

Methods of making allowance for overhead and labour costs of production, or allowing for a minimum profit on the meal fed are described.

It is concluded that meal feeding pays best in the early stages of growth.

The use of meal in times of milk shortage to raise the daily intake to the level of the Ruakura No. 1 ration will improve feed efficiency and increase the monetary returns from the milk fed.

By using meal supplements in the early part of the season, the growth rate of early-farrowed pigs can be maintained so that these animals are large enough to cope efficiently with the increased milk supplies available later in the dairy season.

Introduction

A farrowing schedule designed to provide the best fit of pig feed requirements to the seasonal variation in the separated milk supply on New Zealand farms has been described (Smith, 1956a). As has been pointed out, however, (Smith, 1956b), when such a schedule is put into effect, the food requirements of the pigs on hand at the beginning of the dairy season will exceed the supply of separated milk at that time, with the result that either meal supplements must be used or inadequate rations fed. The consequences of underfeeding are two-fold:

There is a lowering of the efficiency of feed conversion proportional to the reduction in the ration below a recommended level (the Ruakura No. 1 ration—Smith 1956b).

Pigs whose early growth has been limited to that supported by the milk supply of the early part of the dairy season will be too small to consume the increased quantity available later in the year without overfeeding.

With overfeeding, as with underfeeding, the efficiency of feed conversion is lowered.

In this paper, the economics of the use of meal supplements with separated milk will be examined under three heads:

The profitability of meal feeding *per se*.

The effect of meal feeding upon the monetary returns from the separated milk fed in conjunction with it.

The effect of meal feeding during the early stages of fattening upon the feed capacity and feed conversion rate of pigs later in their fattening life.

THE PROFITABILITY OF MEAL FEEDING *Per Se*

Smith (1953) has shown that where meal is used to supplement separated milk, the food value of a pound of meal (one part of 60%-protein meat-meal to three parts barley) and a gallon of milk are practically identical. On this basis, the weight increase obtained by feeding a mixture of milk and meal can be apportioned to the two ingredients of the ration according to the proportion of each used. In this section, the economics of feeding meal will be considered without reference to the separated milk fed with it.

The relationship of meal prices, meat prices, and the feed requirements per pound of carcass gain will determine the economics of meal feeding at any given time and for pigs of a given weight. The price of meal or meat is beyond the control of the farmer and varies over the years. The feed required per pound of gain will vary as a result of both environmental and genetic influences within and between herds but control of these variables is to some extent in the hands of the farmer. The values for feed requirement per pound of live-weight gain used in this paper are those calculated from feed and growth records obtained at the Ruakura Animal Research Station (Smith, 1953) and represent a level of efficiency which is not beyond the average farmer. More recently, breeding stock from 21 pig studs which supply animals to the industry were purchased and their progeny fattened at this Station. The average growth rate and feed efficiency of these animals corresponded very closely to the previous estimates based solely upon Ruakura stock. It is evident, therefore, that fattening pigs in the industry are genetically capable of achieving the standards used in the following calculations:

(a) The feed required to produce a pound of live-weight gain on a pig of any given weight can be calculated from the regression $Y = 0.015x + 1.72$ where Y is the feed required in feed units and x is the live weight of the animal.

(b) The live weight of a pig can be converted to carcass weight by means of the equation $Y = 0.79x - 4.4$ where Y = carcass weight and x is the live weight (Smith, 1957).

(c) Any given live-weight increment can be converted to carcass increment by multiplying by 0.79 (Smith, 1957).

(d) From these equations the feed requirement per pound of carcass-weight gain can be established as $1.26\ (0.015x + 1.72)$.

The food cost in pence of producing a pound of meat on a pig of a given live weight will be $[(0.015x + 1.72)\ 1.26]$ x cost per pound of meal. When this cost equals the price received for meat, meal feeding ceases to show a profit. The important factor in these calculations is the live weight at which costs and returns equalize. Prior to this weight, lower feed costs per pound of gain enable meal to be fed at a profit.

By calculating the cost of producing a pound of meat on pigs of varying live weight at varying meal prices, Fig. 1 was prepared. From this graph, the live weight at which meal feeding ceases to pay can be read directly for any meal : meat price ratio.

If it is desired to maintain a specified profit per pound of meal fed, say 1d. per pound, £8 per short ton should be added to the meal price and the graph read for the appropriate values. On the other hand, if

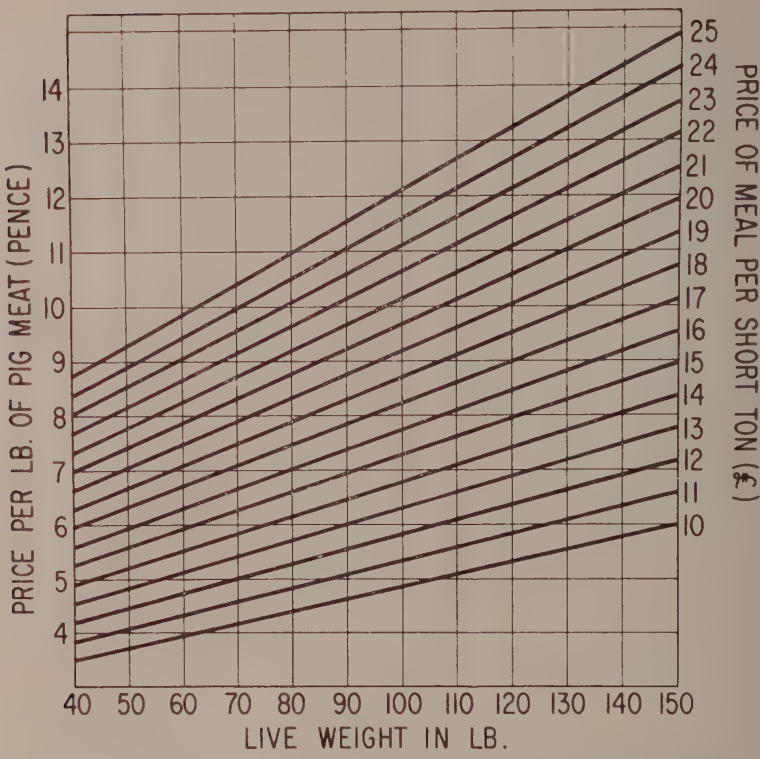

Fig. 1.—The horizontal line starting at the current meat price is followed across the graph until it intersects the sloping line originating at the current meal price. The weight vertically below this point of intersection is the weight at which meal feeding ceases to pay.
Multiples of the values shown may be used as long as both the meat and meal prices are multiplied by the same amount. Thus, the weight arrived at by using the values 4d. for meat price and £10 for meal price are the same as for 8d. and £20.

the piggery overhead costs per pound of meat produced are known, this value can be deducted from the current meat price and the graph read according to the new meat : meal price ratio.

In general, it may be concluded that meal pays best when fed to light weight or young pigs since the feed requirement per pound of gain increases with live weight, and the monetary cost of meat production similarly rises. This is a similar conclusion to that arrived at by McMeekan (1936).

The Effect of Meal Feeding upon the Returns from Separated Milk

When sufficient pigs are weaned in July and August to consume the September and October milk supply, their feed requirements at weaning will exceed the daily farm output of separated milk. These pigs can be fed on a restricted ration or their daily intake can be raised to that of

the Ruakura No. 1 ration by use of meal supplements. Since underfeeding lowers feed efficiency, the economics of the use of meal in times of milk shortage will depend upon two factors:

The extra meat produced from each gallon of milk through increased feed efficiency;

The profit or loss from the meal portion of the ration, which will depend upon the current meal : meat price ratio and the weight of the pigs being fed.

When the results obtained in the feeding-level trial conducted at this Station, namely that a 1% reduction in feeding level will increase the feed requirements by 0.5% (Smith, 1956b), are applied to average pigs the following values can be calculated:

(Pork 1/6d. per lb., meal £34 per ton)

A pig growing from 40 to 60 lb. live weight on a ration of milk alone fed at a level which is 50% of the recommended ration will require

$$\{[0.015(50) + 1.72]\,20\} + \left\{\frac{50 \times 0.5}{100}[0.015(50) + 1.72]\,20\right\}$$

$= 61$ gallons.

The carcass yield from this 20 lb. of live weight would be $0.79 \times 20 = 15.8$ lb. The gross return for 61 gallons of milk fed would, therefore, be 23/6d. or 4.6d. per gallon. If on the other hand, the available milk were supplemented by an equal quantity of meal to raise the daily intake to the Ruakura No. 1 ration, the feed requirement would be (in gallons of milk and pounds meal) $(0.015(50) + 1.72)\,20) = 49$ units. Since half the ration consisted of meal and half of milk, 24.5 gallons and 24.5 lb. of meal would have been used. The gross return would again be 23/6d. of which 11/9d. could be credited to the meal portion of the ration and a similar amount to the milk. The milk fed with supplements would return 5.75d. per gallon or 1.15d. more than the unsupplemented milk. The cost of feeding 24.5 lb. of meal would be 100d. leaving a profit of 41d., or 1.7d. per lb. of meal fed.

If, as is accepted practice in New Zealand, all the profit accruing from meal feeding is credited to the separated milk on the premise that meal feeding is a means of obtaining a full and efficient utilization of milk which is the major feed supply, then the return per gallon of milk fed is 7.4d.—an increase of 2.8d. per gallon through the use of meal.

Where separated milk is credited with the profit from meal feeding, it can in certain circumstances absorb a loss from the same source. This situation may arise toward the end of the dairy season when a decision has to be made between using meal to supplement a falling milk supply in order to finish a late-farrowed litter before the winter, or feeding a falling ration and carrying the pigs through the winter to be fattened the following spring. While the meal fed may return less than its cost in such cases, the increased efficiency of conversion of the ration obtained by providing a reasonable daily intake could offset the loss on the meal part of the ration. In practice, it is probable that less total meal would be required as a supplement to the separated milk to provide a fattening ration than would later be used to provide a maintenance ration for the same pigs over the winter.

The Effect of Meal Feeding During the Early Stages of Growth upon Subsequent Consumption

On an average New Zealand dairy farm, for each gallon of milk produced in July approximately 3½ are produced in August, 6 in September, and 8 in October. As has been pointed out, both pig numbers and pig appetite must be organized to take care of these increases in supply.

Where restricted feeding is practised in July through a reluctance to use meal supplements, both growth rate and feed efficiency are affected. For example, in the experiment at this Station in which levels of feeding were compared, the pigs on the No. 1 ration took 18 days to grow from 40 to 60 lb., and 17 days to grow from 60 to 80 lb. liveweight. Those on 50% of this ration took 43 days and 42 days to make the same gains. At the end of 35 days feeding, therefore, the well-fed pigs were 80 lb. live weight and their poorly fed mates were approximately 56 lb.

In practice, since 3½ gallons of milk are available in August for each gallon produced in July, it follows that a pig receiving one gallon daily when weaned in July must be capable of consuming at least three gallons per day in August. The remainder of the August supply will be eaten by the litters weaned in that month. A pig of 80 lb. live weight will comfortably and economically drink 3 to 3.25 gallons per day, but to reach this weight in August the July milk supply would need to be supplemented by approximately an equal quantity of meal.

The alternative is to feed the inadequate milk ration available during July, and then endeavour to deal with the increased supply in August and September by feeding on an *ad lib.* basis. Work at this Station (Smith, 1956b) has shown that *ab lib.* feeding, as compared with feeding at the Ruakura No. 1 ration level, reduced feed efficiency by approximately 5% and, in addition, induces scouring with a consequent fouling of the pens. It is evident, therefore, that the use of meal supplements during the early part of the dairy season results in better feed utilization during the period of restricted milk supply and produces animals sufficiently large to consume economically the increased supplies during the subsequent months.

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OCCURRENCE OF PEACH CALICO IN NEW ZEALAND

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(*Received for publication, 27 May 1957*)

Summary

A virus disease that causes a brilliant yellow or papery white variegation of leaves has been found on peaches and nectarines in New Zealand. Although widespread, it is present on only a few trees in any district.

Symptoms on leaves, twigs, and fruits, slow movement of the virus through trees, and the limited host range indicate that this virus disease is identical with peach calico described from the United States of America. Although comparatively low in New Zealand, incidence appears to be considerably higher than in the United States, the only other country from which this disease has been described.

Heat treatment of growing plants at 99°F. for 40 days had no effect on the virus.

Introduction

In January 1948, foliage chlorosis was observed on a Goldmine nectarine tree in Central Otago. During the following season somewhat similar symptoms were seen on leaves of a number of nursery trees of the peach variety J. H. Hale in the Waikato. Transmission experiments have shown that this chlorosis is caused by a virus having the characteristics of peach calico.

Symptoms

Most noticeable symptoms are pale-green, cream, bright-yellow, or almost white areas on leaves. There are two types of chlorosis, an irregular blotching (Fig. 1), and a form of vein banding (Fig. 2). In both, chlorosis may become so extensive as to involve almost the whole leaf (Figs. 1e and 2b). Not only does each type of symptom usually remain constant within a tree but it is reproduced when the disease is transmitted to other trees. Some leaves on a predominantly blotched tree may, however, show veinal chlorosis and *vice versa*.

With both blotch and veinal chlorosis, it is not uncommon to find leaves showing three colours, i.e. normal green, pale green, and cream or bright yellow (Figs. 1b and 2a). Margins of areas of different colours are not clearly defined.

Bark of young shoots bearing leaves with symptoms may fail to develop the normal green colour of shaded wood, but exposed surfaces generally show the shade of pink normal for that variety. When leaf symptoms are mild, bark is likely to be a paler green than normal, but where symptoms are severe it may be almost white.

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Fig. 1.—Peach calico on peach.
a–c Leaves from infected J. H. Hale peach.
d & e Leaves from peach seedling stock infected with peach calico ex J. H. Hale peach. × 7/16 (approx.)
Photo: J. W. Endt

Fig. 2.—Peach calico on peach and nectarine.
a & b Leaves from infected Goldmine nectarine.
c & d Leaves from infected Golden Sovereign peach.
× 7/16 (approx.)
Photo: J. W. Endt

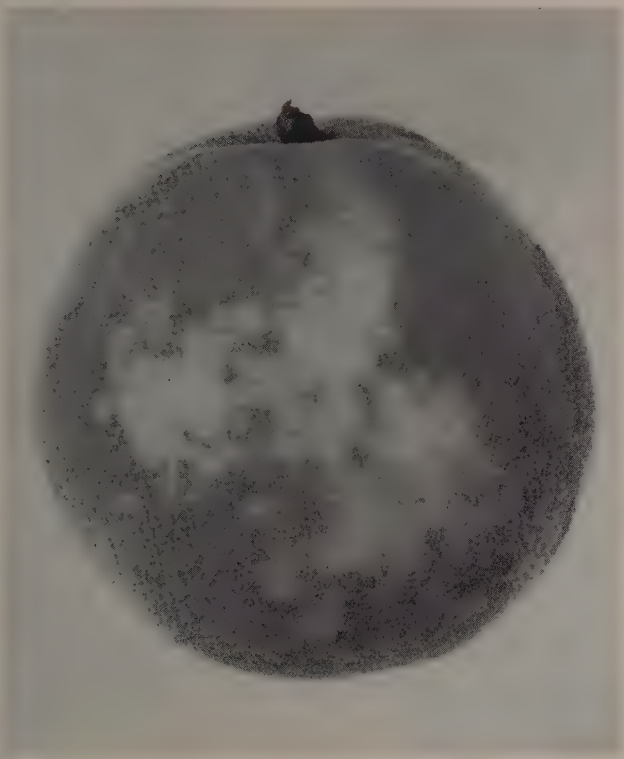

Fig. 3.—Symptoms of peach calico on immature peach fruit var. J. H. Hale. × 4/3 (approx.)

Photo: J. W. Endt

A small percentage of fruit from infected trees may show white blotches on the surface (Fig. 3).

Symptoms are commonly confined to part of a tree (Fig. 4), but occasionally they may develop on all branches. In some trees symptoms of peach calico spread slowly. The amount of chlorosis on infected trees varies widely, irrespective of symptom type. When yellowing is pronounced there is a marked reduction of tree vigour, but when symptoms are mild tree growth is not noticeably reduced.

INCIDENCE AND VARIETIES INFECTED

Peach calico is widely distributed through New Zealand but only a few infected trees have been found in any one orchard. It has been found on ten peach varieties and one nectarine. Details are given in Table 1.

In only one case was incidence high in an established orchard, suggesting that there is little natural spread.

Fig. 4.—Peach calico on peach. Peach seedling stock infected by grafting with bark from infected J. H. Hale peach. Note one shoot showing severe symptoms and one shoot, on right, showing mild symptoms.

Photo: J. W. Endt

Transmission Experiments

Attempts to transmit the disease were made by inserting into healthy stocks buds or pieces of bark taken from infected trees. Scions were allowed to grow and in all cases developed typical symptoms. Attempts to transmit the disease to apricot, cherry, and plum failed. Details of

experiments in which peach calico was transmitted from peach or nectarine to peach seedling stocks are given in Table 2.

TABLE 1. INCIDENCE OF PEACH CALICO ON PEACH AND NECTARINE IN NEW ZEALAND

Variety	Date Observed	No. Trees Infected	Locality (District)	Remarks
		Peach		
J. H. Hale	Dec. 1948	Not recorded	Waikato	Nursery trees
,,	Feb. 1949	1	Waikato	Budwood tree*
,,	Dec. 1949	1	Hawke's Bay	—
,,	Dec. 1949	1	Hawke's Bay	In block of 6 orchard trees
,,	Dec. 1950	2	Central Otago	—
,,	Jan. 1950	4	Hawke's Bay	In block of 28 orchard trees
,,	Dec. 1951	Not recorded	Central Otago	—
,,	Jan. 1953	1	Central Otago	—
,,	Dec. 1953	12	Central Otago	In block of 18 orchard trees
,,	Dec. 1954	14	Wellington	In planting of 200–300 nursery trees
,,	Jan. 1957	2	Hawke's Bay	—
Akarana	Jan. 1950	1	Hawke's Bay	In block of 30 orchard trees
Briggs	1953	Not recorded	Central Otago	—
Golden Queen	Jan. 1950	1	Hawke's Bay	—
Golden Sovereign†	Mar. 1950	2	Canterbury	—
Kahuranaki	Jan. 1950	1	Hawke's Bay	—
,,	Jan. 1950	3	Hawke's Bay	In block of 25 orchard trees
Paragon	Dec. 1949	1	Hawke's Bay	—
,,	Nov. 1956	1	Auckland	In block of 18 orchard trees
Stark	Not recorded	1	Hawke's Bay	—
Wiggins	Mar. 1950	Not recorded	Canterbury	—
Unidentified Var.	Dec. 1953	2	Central Otago	—
,,	Nov. 1956	2	Manawatu	Nursery trees
		Nectarine		
Goldmine	Jan. 1948	1	Central Otago	—

* Budwood from this tree was used to propagate the nursery trees recorded as infected in December 1948.

† "Golden Sovereign" is thought to be a local name for this late, white-fleshed variety.

TABLE 2. TRANSMISSION OF PEACH CALICO FROM PEACH AND NECTARINE TO HEALTHY PEACH SEEDLING STOCKS

Infected Scion Variety	Locality*	No. Stocks Budded	No. Scions Growing	No. Stock Showing Infection after			
				1 yr.	2 yr.	3 yr.	4 yr.
Peach							
J. H. Hale	Waikato	6	4	0	0	0	
J. H. Hale	Waikato	5	5	1	5		
J. H. Hale	Waikato	12	12	7	10		
J. H. Hale†	Waikato	13	—	2	12		
J. H. Hale	Hawke's Bay	2	2	0	0	1	2
Golden Sovereign	Canterbury	2	2	0	0	0	2
Golden Sovereign	Canterbury	6	4	1	2	2	
Kahuranaki	Hawke's Bay	6	3	0	0	2	3
Briggs	Central Otago	3	2	0	0		
Nectarine							
Goldmine	Central Otago	3	3	0			
Goldmine	Central Otago	6	6	0	1	1	

* In this column is given the locality from which the original infected material was obtained.

† In this trial pieces of bark from infected trees instead of buds were inserted in the healthy stocks.

Results show that the disease was successfully transmitted from five distinct sources.

Transmission was obtained in two other experiments. In one, a small wedge of wood from an infected Goldmine nectarine tree was inserted into a healthy peach stock, and then covered with a bud from a healthy Goldmine. Of 20 trees worked in this way 14 produced Goldmine scions, four of which showed peach calico in the year following budding. In the other experiment, two healthy buds of the indicator variety Elberta were inserted into a peach stock already supporting an infected J. H. Hale scion. Peach calico spread to one Elberta scion in the year following budding.

Rate of Spread Within a Tree

Movement of peach calico within naturally infected trees is slow, and the following observations made during transmission experiments confirm this:

Although symptoms appeared on infected scions in the year following budding, one to four years elapsed before they appeared on the stocks.

When symptoms did appear on stocks they invariably appeared first on shoots growing in close proximity to an infected scion.

In several instances the proportion of stock shoots showing symptoms became progressively greater each year after infection.

EFFECT OF HIGH TEMPERATUBE ON PEACH CALICO

Four small J. H. Hale trees infected with peach calico were held for 40 days at an air temperature of 99°F. (± 2°F.), soil temperature being on an average 3° to 4°F. below air temperature. On removal from high temperature conditions, trees were planted in the open. In the following spring all four trees showed severe symptoms on new shoots, indicating that heat treatment had not eliminated this virus.

IDENTITY

Three diseases of peaches, each bearing some resemblance to the disease described in this paper have been recorded in North America. These are peach variegation, a bud sport (Blodgett, 1944 and 1951), peach calico, a virus disease (Blodgett and Reeves, 1951), and peach blotch, also a virus disease (Willison, 1946).

The New Zealand form of chlorosis is transmissible and therefore of virus origin. Although it has some features in common with peach calico and peach blotch, it appears to be much closer to the former. Peach calico and the New Zealand disease both cause brilliant yellow to papery white blotches on foliage, and chlorosis of twigs and fruit. Both are difficult to transmit, appear to move slowly within trees, and may be found on isolated branches. They differ from peach blotch, which causes a pale-green to yellow-green blotching of leaves and scorching of leaf margins, but does not produce chlorosis of twigs or fruit.

On this evidence it is considered that the New Zealand disease is caused by the same virus as peach calico of North America.

DISCUSSION

Since the known incidence of this disease is restricted to a total of 11 trees in the United States of America (Blodgett and Reeves 1951; Stout, 1952), and some 50 to 60 trees in New Zealand, and since there is no evidence of rapid field spread, it is unlikely that peach calico will become a serious disease.

ACKNOWLEDGEMENT

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A SHINY-PODDED MUTANT IN POLE BEAN (*Phaseolus vulgaris* L.)

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(*Received for publication, 16 April 1957*)

Summary

A mutant in pole bean causing shiny pod surface is described.

Crosses between the mutant type, the parent variety Fardenlosa, and another variety, the rust-resistant Westralia, as well as data from F1, F2, and backcrosses to the mutant, show that the condition is inherited as a single recessive gene, being expressed in homozygotes.

No linkage between pod type and rust reaction could be detected. The new gene has been called *ace*.

Introduction

The mutant in pole bean to be described here, was first noticed and propagated by a market grower in Auckland, New Zealand. It was found to have commercial value owing to its distinctive appearance as a fresh bean, and was claimed to have keeping qualities superior to the normal pod type. This claim has not been substantiated, but the shiny pod does confer an *appearance* of freshness after keeping, which retailers and consumers prefer, as shown by the fact that it has enjoyed a premium over the normal type in the markets.

The parent of the mutant is a variety known as Fardenlosa, which is the most commonly grown pole bean in New Zealand, and is said to be of German origin. The following is a brief description:

Plants dark green, vigorous climbers, reaching the top of 7-ft. stakes and often trailing above; stems medium thickness; flowers white, commonly 7 or 8 per stem; pods 7–9 in. in length, medium dark green, shape similar to Kentucky Wonder, but generally straighter; seed white, rounded ends, 125 per ounce.

The mutant has not been described hitherto, but its value as a commercial type, and the possible use of the gene concerned as a marker have prompted its description and inheritance study.

Description of Mutant

Plants of the mutant conform in all respects to the descriptions of the parent variety except for the appearance of the pod. Fig. 1 shows the shiny character of market-size pods in comparison with the normal dull surface common to most green bean varieties. Lamprecht (pers. comm.) has compared its appearance with that of some segregates from a *P. vulgaris* × *P. coccineus* cross which he has not been able to obtain in a pure-line. Occasionally, individual shiny pods are observed on *P. vulgaris* plants bearing the normal dull pods, but these do not breed true, and are regarded as due to rubbing-off of the waxlike bloom on stakes. The appearance of this shiny pod can be likened to the "emerald" mutation in peas although, in the latter the whole plant is affected (Vilmorin, 1910; Tschermak, 1912).

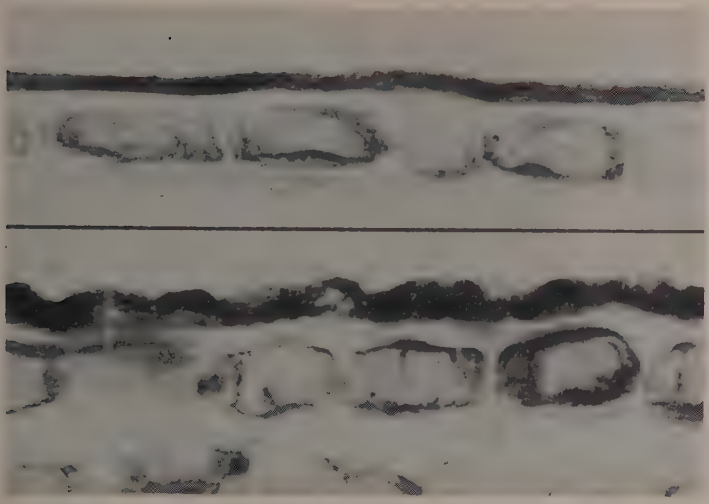

Fig. 1.—*Upper:* Shiny-podded mutant. *Lower:* Fardenlosa.

Photo: S. A. Rumsey.

Fig. 2.—*Upper:* Cross-section of fully grown green bean of the mutant type, showing relatively flat-surfaced cuticle.
Lower: Parent type, with thicker crenate cuticle presenting a surface unfavourable to light reflection. × 348.

Photo: S. A. Rumsey.

Separation of plants with dull and shiny pods is best done when the first pods are five inches or so in length. Earlier than this, observations are apt to be inaccurate. Soon after petal fall, when the

pods are 1–2 in. long, both types have a shiny appearance, and at 3–4 in. potentially dull pods often still retain the shiny character, although on close inspection the extremities of the pods are found to have dull areas.

The structural differences between the two pod classes can be seen in microphotographs of portions of cross-sections of mature pods (Fig. 2). The cuticle of the shiny pods, when freshly cut sections were stained with Sudan III, was observed to be a smooth layer, the upper surface being entire. The dull pods under similar treatment showed a cuticle whose upper surface was irregularly crenate in appearance. It would appear that a greater quantity of cutin is produced in the dull-podded type and that the irregular surface caused by the piling-up of the cutin does not present a light-reflecting surface comparable to that given by the smooth cuticle of the shiny-podded type.

Breeding Tests

When samples of the shiny-podded bean seed obtained from market gardeners have been grown, there has usually been a proportion of dull-podded plants present so that there has been some doubt about the constancy of the shiny character. Over four and five generations of selfing, after selection of single plants, the shiny character was stable throughout the single-plant lines. From this material, pure-line seed has been released for commercial planting (Yen, 1957).

The dull-podded impurities in market gardeners' seed must then be due to inefficient isolation at flowering time and harvest.

Crosses were made between mutant and type, using the pure lines referred to above and the parent type Fardenlosa (Cross 1), as well as the rust-resistant Westralia, another pole bean type (Cross 2). Reciprocal crosses were made, but these have not been separated in the data presented since no differences were observed. Backcrosses to the mutant type were made after observations were completed on the first pods of F1 plants.

All plants derived from Cross 1 were sown outdoors and trained on individual stakes for easy recording. In Cross 2, the F1 and F2 were grown likewise, but the backcross material was subjected to glass-house testing for susceptibility to rust caused by *Uromyces appendiculatus* (Pers.) recorded in New Zealand by Brien and Jacks (1954). The method of inoculation was similar to that described by Harter and Zaumeyer (1941) and observations of resultant rust symptoms were made about 14 days after inoculation.

F1, F2, and Backcross Results

54 F1 plants of both crosses produced pods all conforming to type, indicating that the shiny-podded character is recessive. Seed was saved for F2 observations. F2 data on the two crosses are shown in Table 1, and segregations for pod type conform to a ratio of 3 dull: 1 shiny.

Results of observations from backcross progeny in both crosses are given in Table 2 and these confirm that there is a monofactorial difference between type and mutant.

Gene Symbolization

Lamprecht (pers. comm.) has suggested symbolizing the new gene as *ace* from the Latin a = negative, cera = wax giving the following

TABLE 1. F2 DISTRIBUTION FROM CROSSES BETWEEN DULL- AND SHINY-PODDED BEAN PLANTS

Cross No.	Parents	Total Plants	F2 Plants		χ^2	P
			Dull Pod	Shiny Pod		
1	Fardenlosa × Fardenlosa shiny-podded mutant	373	287	86	0.752	0.50–0.30
2	Westralia × Fardenlosa shiny-podded mutant	297	227	70	0.324	0.70–0.50
	Total	670	514	156	1.053	0.50–0.30

TABLE 2. DISTRIBUTION FROM BACKCROSSES BETWEEN F1 AND SHINY-PODDED PARENT

Cross No.	Total Plants	Plants from Backcross		χ^2	P
		Dull Pod	Shiny Pod		
1	50	26	24	0.080	0.80–0.70
2	215	114	101	0.786	0.50–0.30
Total	265	140	125	0.849	0.50–0.30

genotypes and phenotypes: *Ace Ace,* dull pod; *Ace ace,* dull pod; *ace ace,* shiny pod.

Linkage of Pod Type and Rust Reaction

Data on pod type in the backcross population of Cross 2 are shown again in Table 3, in combination with observations on rust reaction. The New Zealand race or races of rust used for inoculation have not

TABLE 3. POD TYPE AND RUST REACTION FROM BACKCROSS OF CROSS 2, (Ace/ace, R/r) TO SHINY-PODDED MUTANT (ace/ace, r/r)*

	Rust Resistant R/r	Rust Susceptible r/r	Total
Dull-podded Ace/ace	54	60	114
Shiny-podded ace/ace	62	39	101
Total	116	99	215

Segregation Ace, ace	$\chi^2 = 0.786$	$P = 0.50–0.30$
Segregation R, r	$\chi^2 = 1.344$	$P = 0.30–0.20$
Joint segregation	$\chi^2 = 3.912$	$P = 0.05–0.02$

* R, r are symbols used here for rust reaction genes but are not used in bean genetics. No symbolization has been ascribed to genes which confer resistance to various bean-rust races (Zaumeyer and Harter, 1941).

been identified, but rust resistance would appear to be due to a dominant single factor. Zaumeyer and Harter (1941) have reported resistance to some races to be inherited similarly.

Ten plants additional to the number appearing in Table 3 were recorded as rust susceptible, but are not included since they succumbed to the effect of disease before pods could be formed.

From these data there is no evidence of linkage between the pod-type and rust-reaction characters.

Acknowledgements

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EFFECTS OF INGESTION OF PARAFFINS BY RUMINANTS

I. THE EFFECTS OF DIFFERENT LIQUID PARAFFINS ON THE FEED INTAKE OF NON-LACTATING MONOZYGOTIC TWIN COWS

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Summary

The effects of four different liquid paraffins on the feed intakes of non-lactating, stall-fed, monozygotic twin cows have been investigated.

Single doses of 200 ml. of heavy or of light liquid paraffin of medicinal (British Pharmacopoeia) quality were without deleterious effect on the appetite of treated cows.

Temporary reductions of 40–90% in feed intake occurred following a single dose of 200 ml. of an odourless kerosene or of a very light grade of paraffin of high isoparaffinic content. There was a delay between dosing and the appearance of the effect. The length of this delay and the depression of the appetite varied from twin set to twin set, members of an individual set responding alike. Recovery of appetite was progressive, taking usually 3–4 days. The loss in intake was not made up subsequently.

The time lag was almost abolished and the degree of appetite depression was approximately doubled by introducing 200 ml. of coarsely emulsified odourless kerosene directly into the omasum. From this and other evidence, it is considered that the initial stage at least, of the effect is post-ruminal.

Possible evidence of activity of the oesophageal-groove reflex in one pair of adult twin cows was obtained.

It is concluded that it is undesirable to administer very light paraffins to ruminants. The significance of this is discussed in relation to treatment and prevention of bloat.

Attention is drawn to the value of identical twins for experiments on factors affecting the appetite.

Introduction

The use of anti-foaming agents in the treatment and prevention of frothy bloat in sheep and cattle has been investigated by a number of workers (Clark, 1948, 1950; Quin *et al.*, 1949; Johns, 1954; Ferguson and Terry, 1955; Barrentine, 1957; Reid and Johns, 1957). Reid and Johns found the most reliable materials to be oily or fatty substances, and recommended the use of peanut oil or emulsified mixed ruminant tallow, because these have little or no effect on milk and butterfat production or quality (McDowall, Patchell, and Reid, in preparation), and appeared unlikely to have any deleterious effects on animal health.

Reid and Johns investigated a range of paraffins from low-viscosity kerosene to wax emulsions. Liquid paraffins were as effective as vegetable oils in relieving bloated animals and, since they are cheaper, appeared to be an attractive alternative for use in the field. However, in non-ruminants the ingestion of mineral oils may result in undesirable side effects. Continued absorption of small amounts of paraffin can result in chronic inflammation of the intestinal walls and other organs (Stryker,

1941). Because it does not elicit the cough reflex, mineral oil may be aspirated into the lungs, where it may cause "lipoid" pneumonia and fibrosis (Schneider, 1949; Meyers and Griffith, 1955). Finally, unabsorbed paraffin entrains a proportion of the fat-soluble materials in the diet, including carotene and vitamins A, D, E, and K (McDowall *et al.*, 1957), which become lost to the animal when the oil is excreted in the faeces.

The first two of these effects are usually associated with prolonged ingestion of paraffin and do not seem likely to be important here. However, the effect on the fat-soluble constituents of the diet is more immediate and may have serious consequences such as interference with the retention of calcium and phosphorus (Smith and Spector, 1940 a, b). As no information on the effects of paraffin ingestion by ruminants has been published, it was considered necessary to determine whether these harmful actions were significant at the dose rates normally used for bloat control, before the mineral oils could be recommended for field use.

A number of experiments have been carrid out and the results will be presented in this and succeeding parts of a series. This paper reports the effects of different liquid paraffins on the appetites of dry cows. The following paper (McDowall *et al.*, 1957) gives the results of a drenching trial with heavy liquid paraffin given to milking cows, and its effects on the quality of milk and butterfat and the absorption of carotene and vitamin E. Further experiments on the effect on carotene absorption are reported in the third paper (McGillivray, 1957).

During their investigation of the effectiveness of various paraffins in the treatment of bloated cows, Reid and Johns (1957) observed that the very light liquid paraffins appeared to depress the appetite temporarily. This paper reports some experiments made on the effects of four different grades of liquid paraffins on the feed intake of stall-fed, dry, identical-twin cows.

Methods

Animals

Three sets of dry, monozygotic-twin dairy cows were used, numbers 47 and 48, 89 and 90, and 293 and 294. Cows 48, 293, and 294 had rumen fistulae closed by rubber cannulae and bungs of the type described by Balch and Johnson (1948). The animals were accustomed to being handled, each set having had at least one season's experience of stall-feeding before the present experiments were commenced.

Feeding

The animals were stall-fed with fresh herbage cut from almost pure stands of red clover. The feed was given *ad lib.* in two two-hour periods, one in the morning, the other in the afternoon. Between feeds and overnight, the cows were held in a bare yard, with access to water. This feeding regime, which has been used for the past four years on this station, is described in detail by Johns (1954).

The feed offered was weighed at the commencement of and at 15 min. intervals throughout each two-hour feeding period. At all times care was taken to offer adequate feed in a palatable condition. The feed boxes were frequently "topped up" during a feeding period. If rank

weeds or soiled clover began to accumulate, the boxes were emptied and entirely fresh feed was offered.

Stall-feeding was carried out for periods of 5–7 days. On days when they were not fed in the stalls, the cows grazed a mixed pasture of white clover, red clover, and ryegrass. They were brought back into the holding yard on the evening of the day before recommencement of stall-feeding.

Materials

Four paraffins were used:

1. Heavy liquid paraffin, British Pharmacopoeia grade (White Oil 890*).
2. Light liquid paraffin, B.P. (Oil S-1020*).
3. Odourless kerosene (EF471W*).
4. A very light liquid paraffin containing a high proportion of iso-paraffins (Stanvac Odourless Solvent*).

The characteristics of these materials are set out in Table 1.

TABLE 1. CHARACTERISTICS OF PARAFFIN SAMPLES

	White Oil 890	Oil S-1020	EF471W	Stanvac Odourless Solvent
Specific Gravity (20°c.)	0.880	0.852	0.782	0.788
Viscosity (centistokes: 37.8°c./37.8°c.)	74	17	2.4 (approx.)	2.2 (approx.)
Volatility				
Initial Boiling Point (°c.)	—	—	201	204
Final Boiling Point (°c.)	—	—	264	256
Vapour pressure at 37.5°c. (mm.Hg)	Very low	Less than 1	5 (approx.)	5 (approx.)
Percentage unsulphonatable	99	99	97	97
Fluorescence (Woods filter)	Trace	Trace	+ (Lilac)	+ (Lilac)
Odour	Nil	Nil	Faint oily smell	Nil
Taste	Nil	Nil	Mild flavour of Kerosene	Mild flavour of Kerosene

White Oil 890 and Oil S-1020 meet the B.P. and U.S.P. tests which set limits to acidity, carbonizable (sulphonatable) substances, solid paraffins, and sulphur compounds.

* Code names of products of the Vacuum Oil Co., N.Z. Ltd., who generously supplied the materials.

Treatment Procedure

The animals were stall-fed for at least one complete day before being given paraffin. Treatments were carried out immediately before a morning feed. Only one member of a twin set was dosed in any particular trial, the untreated mate being used as a control. The interval between dosing (1 week) was sufficient to allow complete recovery of intake.

The day of treatment was designated day 0, pre-treatment days as −1, −2, etc., and post-treatment days, as 1, 2, 3 and so on.

The paraffins were administered as drenches except in Exp. 3. The dose used in all experiments was 200 ml. Except in the cases where emulsions were given, the paraffin was shaken with about 300 ml. of water immediately before drenching.

EXPERIMENTAL

The Use of Monozygotic Twins for Experimenting with Factors Affecting Appetite

The appetites of the members of a set of monozygotic twins kept together, fed alike, and in the same physiological state, are closely similar. This is shown by the similarity of the intakes of palatable feed of stall-fed dry twins and by the twin efficiency values of 72 for grazing time (Hancock, 1950) and 8 for D.M. intake (Hancock, 1952) found for free-grazing lactating twins at the same stage of lactation. Monozygotic twin mates also show likeness in their reactions to unpalatable feeds and in the occurrence of abnormal behaviour such as suckling and earth-eating.

The similarity of intake of the twins under the stall-feeding regime used here is illustrated by the dry matter (D.M.) intakes of cows 89, 90 and 293, 294 during a period in which no treatments were given (Fig. 1a, b). Further examples are provided by the intake curves for the trial with heavy liquid paraffin, which had no effect on the intakes of the treated animals (Fig. 1c, d: see Exp. 1). Table 2 shows the cumulative D.M. intakes over the period of this trial.

It will be seen that the cumulative intakes of members of a set show close concordance, but the rates of cumulation are different for the two sets.

The difference in D.M. intake between members of a stall-fed twin set for individual feeds has usually been less than 0.5 kg. The average within-set differences for the feeds shown in Fig. 1a and b were 0.38 kg. for cows 89, 90 and 0.40 kg. for cows 293, 294. The corresponding averages for the feeds in Fig. 1c and d are 0.22 kg. and 0.30 kg. These figures compare favourably with the figure of 0.40 kg. given by Hancock (1952) for 12 sets of grazing identical twins. Over short periods, the differences appear to be random.

In the present experiments, advantage has been taken of the similarity of intakes of identical twins to show the effects of treatments by expressing the intake of the treated animal as a percentage of the intake of the untreated (control) twin. This allows the results of a twin experiment to be presented as a single curve, facilitating comparison of

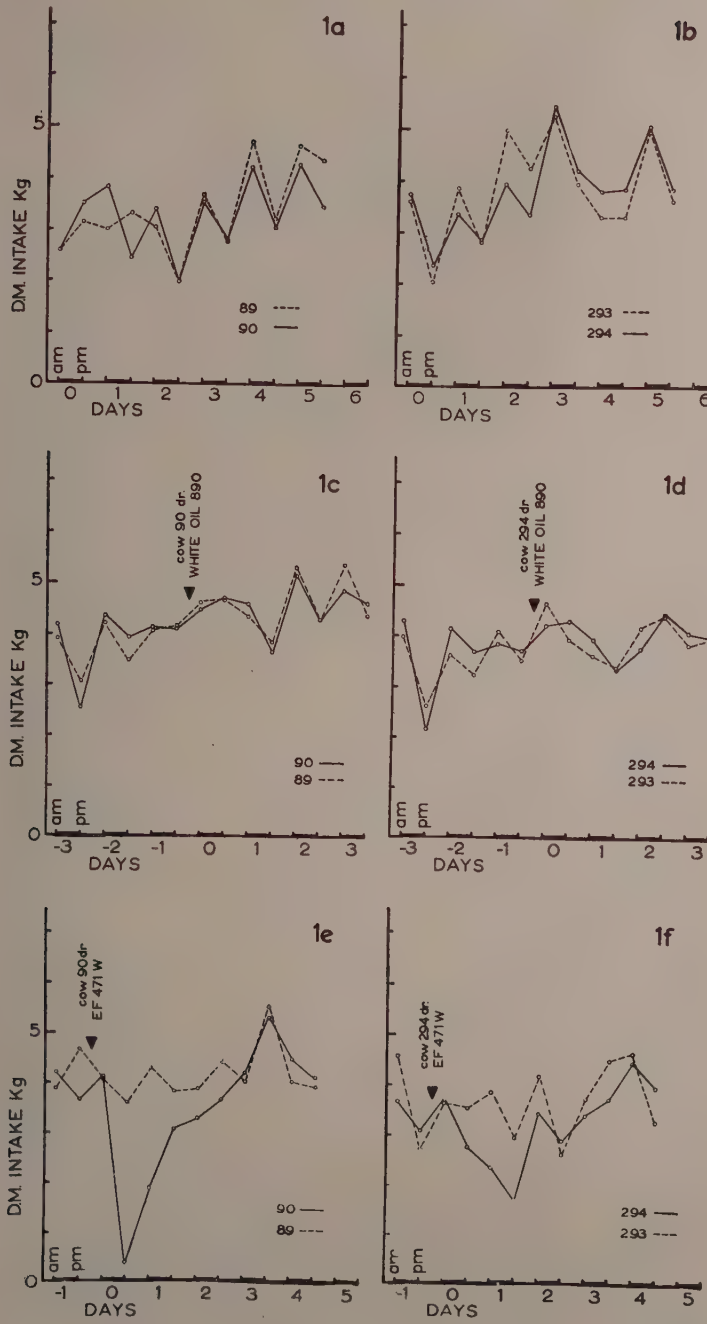

Fig. 1.—a, b.—Typical D.M. feed-intake curves for twin sets 89, 90 and 293, 294 (no treatments).
c, d.—Effects of heavy liquid paraffin B.P., and
e, f.—Effects of odourless kerosene, on the D.M. intakes of these animals.

TABLE 2. CUMULATIVE D.M. INTAKE OF TWIN SETS 89/90 AND 293/294 OVER ONE WEEK (Kg.)

Date	Feed	Cows			
		89	90	293	294
4.2.56	a.m.	3.87[1]	4.12	3.94[1]	4.26
	p.m.	6.91	6.64	6.53	6.37
5.2.56	a.m.	11.07	10.94	10.12	10.48
	p.m.	14.52	14.84	13.31	14.15
6.2.56	a.m.	18.54	18.94	17.39	17.96
	p.m.	22.67	23.02	20.89	21.64
7.2.56	a.m.	27.27	24.47[2]	25.50	25.81[2]
	p.m.	31.90	32.13	29.40	30.12
8.2.56	a.m.	36.22	36.69	32.98	34.00
	p.m.	40.04	40.31	36.35	37.32
9.2.56	a.m.	45.33	45.42	40.47	41.03
	p.m.	49.61	49.69	44.84	45.45
10.2.56	a.m.	54.95	54.55	48.67	49.50
	p.m.	59.29	59.15	52.65	53.44
Cumulative within-set difference (after 14 feeds)		0.14		0.79	
Cumulative between-set difference (average after 14 feeds)		6.18			

[1] 89, 293 were drenched with 200 ml. of Stanvac Odourless Solvent before a.m. feed on 31.1.56.

[2] 90, 294 drenched with 200 ml. White oil 890 before a.m. feed 7.2.56.

the effects of different treatments in one figure (see Fig. 2, 3). It will be seen from Fig. 2a, that the normal range of differences of intake between twins is approximately ± 10% when expressed in this manner.

Monozygotic twins appear to be valuable subjects for the investigation of the difficult problem of ruminant appetite. It is not claimed that the technique used here is ideal. Clearly, experiments must be replicated to detect small differences. Larger numbers of sets would be required to obtain any indication of the full range of reactions. Finally, it is inherent in the use of standardized diet that alterations in the pattern of appetite are not detected.

Experiment 1

The first experiment consisted of trials of the four paraffins listed above. Two pairs of twins, 89 and 90, 293 and 294, were treated according to the schedule shown in Table 3.

The effects on D.M. intake of the treatments with heavy liquid paraffin and odourless kerosene are illustrated in Fig. 1c–f, while in Fig. 2,

TABLE 3. TREATMENT SCHEDULE—EXPERIMENT 1

Date of Treatment	31.1.56	7.2.56	14.2.56	21.2.56
Treatment	Odourless Solvent	Paraffin Heavy Liquid B.P.	Odourless Kerosene	Light Liquid Paraffin B.P.
Cows treated	89 293	90 294	90 294	89 293
Control cows	90 294	89 293	89 293	90 294

the results of all treatments are shown by expressing the intakes of treated animals as a percentage of the intakes of their untreated twin mates.

Neither the heavy nor the light liquid paraffins of medicinal quality appeared to have any deleterious effect on feed intake.

In contrast, both the odourless kerosene and Stanvac Odourless Solvent caused a marked depression of feed intake. There were differences between twin sets both in the degree of depression and the time taken to attain maximum depression. These differences were most marked following treatment with the odourless kerosene (Fig. 2d). At the times of maximal depression, the intake of cow 90 was only 9.7% of that of cow 89, whereas the intake of cow 294 was 57.5% of that of 293, these points occurring on the afternoons of day 0 and day 1 respectively. The effects of both paraffins appeared to have worn off by day 2 or 3.

The trial with Stanvac Odourless Solvent was complicated by the occurrence of bloat in the control (untreated) twins on the mornings of days 1, 2, and 3. In each case, the bloated animals were relieved with a drench of 100 ml. of peanut oil, which was known not to depress the feed intake (unpublished results). Bloating interfered more severely with the intake of cow 294 than with that of cow 90. The effect on 294 was usually to halt feeding. Feeding was resumed as bloat was relieved, but because the feeding period was limited to two hours, the loss of intake could not be made up. This resulted in the intake of the paraffin-treated cow being proportionally higher on the mornings of days 2 and 3 (Fig. 2c) than if the control animal had not bloated. It probably also accounted for the low percentages on the afternoons of those days, when the intake of 294 appeared to be increased, presumably to compensate for the mornings' events.

Although the treated cows often ate more than the control cows for single feeds during the recovery period, it appeared that losses of intake due to paraffin treatments were not made up.

At the time when the concentration of paraffin in the rumen could be expected to be highest, that is, on the morning of treatment, neither the odourless kerosene nor the odourless solvent had any apparent effect on the feed intake (Fig. 2c, 2d). It seemed likely, therefore, that the site of action of the paraffins was post-ruminal. If this were true, then:

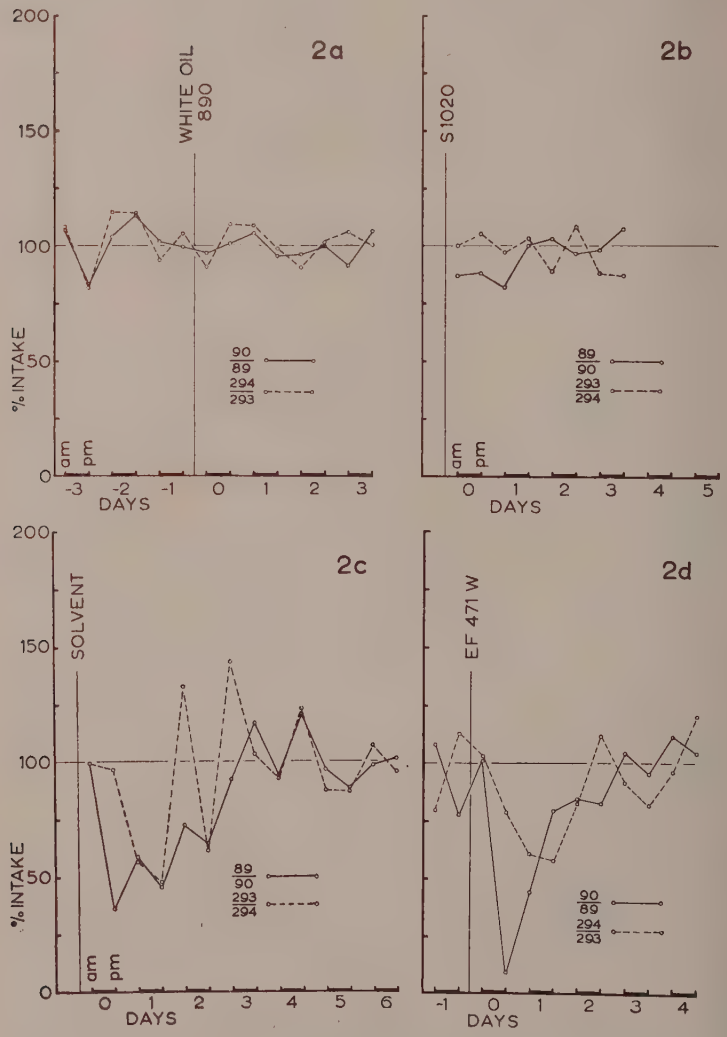

Fig. 2.—Feed intakes of treated cows, expressed as percentages of intakes of untreated twin mates—Exp. 1. All treatments (a drench of 200 ml. of paraffin in each case) given before the morning feed of day 0. (a) Heavy liquid paraffin B.P. (cows 90, 294 drenched). (b) Light liquid paraffin B.P. (cows 89, 293 drenched). (c) Stanvac Odourless Solvent (cows 89, 293 drenched). (d) Odourless kerosene (cows 90, 294 drenched).

(1) delaying the passage of the paraffin through the rumen should delay the effect on intake, and

(2) bypassing the rumen should hasten the effect.

Attempts to test these hypotheses were made in Exp. 2 and 3.

Experiment 2

It is believed that unemulsified oils at dose rates of 200 ml. were substantially lost from the rumen in 3–4 hr. (Reid and Johns, 1957). A method of delaying this loss appeared to be by emulsification of the paraffin to allow greater dispersion through the rumen contents. An emulsion of the odourless kerosene was therefore prepared by mixing for 5 min. in a Waring Blendor 200 ml. of the kerosene, 30 ml. Lissapol N380*, 30 g. Lubrol W*, and 400 ml. of water. This was given as a drench to cow 89. The percentage-intake curve is shown in Fig. 3a, together with that when a drench of unemulsified odourless kerosene was given.

Emulsification failed to delay the onset of the effect: if anything it advanced it, since the intake of 89 on the morning of treatment was only 60% of that of 90. However, there was an increase in the persistence of the depression. Soft faeces were passed immediately after the end of the afternoon feed on day 0, and during the morning feed of day 1. Mild or moderate bloat occurred in cow 90 on the mornings of days 0–3 and was relieved with drenches of 100 ml. soya bean oil.

Experiment 3

Bypassing the rumen was accomplished by intubating the omasum. A rubber tube was introduced about 10 cm. into the omasum of cow 293 and a temporary coarse emulsion of odourless kerosene, prepared by mixing 200 ml. of the kerosene and 300 ml. of water in a Waring Blendor for 5 min., was run in over a 5-min. period. The object of emulsification was to dilute the kerosene and to ensure some dispersion in the alimentary tract. The percentage intake curve is shown in Fig 3b, together with the curve when odourless kerosene was given by mouth.

The introduction of the paraffin directly into the omasum caused the depression of intake to occur sooner than when the kerosene was given by mouth, and increased the severity of the effect. On the morning of treatment, the percentage intake of 293 was only 44.4% of that of its untreated twin 294. The maximum depression occurred on the afternoon of day 0 as against the afternoon of day 1 when the paraffin had been given by mouth, and the lowest intake was 14.2% as against 57.5%. Soft faeces were passed after the afternoon feed of day 0 and again after the morning feed of day 1. Firm faeces were observed on the afternoon of day 2.

Bloat occurred in the control animal on the morning of days 0–3 and on the afternoon of day 1. Drenches of 100 ml. of soya-bean oil were given. On the morning of day 1 bloat markedly reduced the intake of 294. As a result the percentage intake figure for 293 was some 20% higher than it would have been had the control animal not bloated.

Kymographic records were made of the pressure changes in the dorsal rumen of cow 293 during each feed for the period of this experiment, using a balloon and a bellows manometer. There was some

* Non-ionic surface active agents (polyethylene glycol derivatives) manufactured by Imperial Chemical Industries.

irregularity of activity on the morning of treatment, but otherwise the patterns were normal throughout.

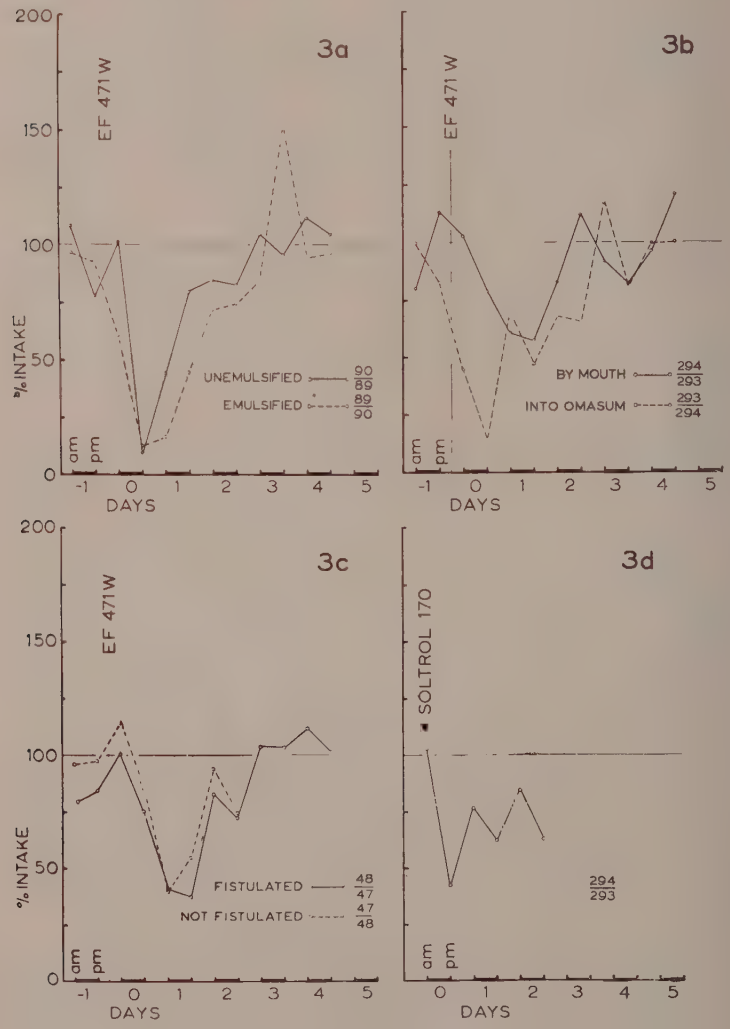

Fig. 3.—Feed intakes of treated cows, expressed as percentages of intakes of untreated twin mates. (a) Comparison of the effects of drenches of plain and of emulsified odourless kerosene (Exp. 2). (b) Comparison of the effects of odourless kerosene given by mouth, and by intubation of the omasum to bypass the rumen (Exp. 3). (c) Comparison of the reactions of a cow with a rumen fistula and of its intact twin mate, to a drench of odourless kerosene (Exp. 4). (d) Effect on feed intake of a drench of 100 ml. of Soltrol 170—given to 294 to relieve bloat during the morning feed of day 0.

Experiment 4

Since cows 293 and 294 possessed rumen fistulae and 89 and 90 did not, the possibility existed that in some way the differences in reaction to treatment with paraffins were a consequence of fistulation. To test this possibility, a third set of twins was used, cows 47 and 48, only one of which (48) was fistulated. Two experiments were carried out two weeks apart, following the procedure in Exp. 1. In the first, cow 48 was drenched with 200 ml. of the odourless kerosene and in the second, this kerosene was given to cow 47.

As will be seen from Fig. 3c, the reactions of the two animals were similar both as to the degree of depression and the time sequence. Both animals passed semi-liquid faeces on the morning of day 1.

It was concluded that fistulation did not alter the expression of the effects of paraffin treatment.

DISCUSSION

In these experiments, whereas treatment with liquid paraffins of medicinal quality was without deleterious effect on the feed intake of cows, a marked depression of appetite was caused by the same dose (200 ml.) of odourless solvent or odourless kerosene. The results of Exp. 3, in which the rumen was bypassed, indicate that the initial stage, at least, of the effect was post-ruminal.

There were marked differences in the reaction of cows 89 and 90, as compared with cows 47, 48, 293, and 294, to the low-viscosity paraffins, both as to the degree of depression and the time at which the maximum depression occurred. These differences were not a consequence of fistulation. The fact that in cow 293 the magnitude of the depression was increased and the delay in onset almost abolished by bypassing the rumen, suggests that the differences between the twin sets could be due to differences in the rate of passage of the paraffin through the rumen. The results of Exp. 2 also support this hypothesis, since emulsification prolonged the effect of the paraffin, although it failed to delay the onset of the depression.

It is difficult to see how consistent differences in the rate of loss of oils from the rumen could arise in the normally functioning rumen. However, the general similarity of the percentage-intake curve for the experiment in which the rumen was bypassed (Fig. 3b) to that when the odourless kerosene was given by mouth to cow 90 in Exp. 1 (Fig. 2a), suggests the possibility of a partial closure of the oesophageal groove in the latter animal during drenching. Support for this is given by the facts that the onset of the depression was very similar in Exp. 2 and 3 and that both treated animals in these experiments passed semi-liquid faeces 5 to 6 hours after treatment. If the oesphageal groove were closed at the time of drenching, the concentration of paraffin in the part of the alimentary canal beyond the rumen would reach higher levels than those produced by dosing into the rumen, although for shorter periods, and the peak concentration would occur earlier. Consistent activity of the reflex would have to be inferred, since the maximum depression of appetite of cows 89 and 90 occurred consistently on the afternoon of the day of treatment (Fig. 2c, 4a). However, closure of the groove could

be only partial, or vary with circumstances, since oil drenches are equally as effective for relieving these as they are for other cows.

If true, this explanation is of some interest. Activity of the oesophageal groove is considered normally to be lost, or at least inhibited, by the time cattle reach 2 years of age, unless a conditioned reflex has been established and maintained by training the adult animal to continue suckling (Comline and Titchen, 1951). However, cows 89 and 90 have no history of suckling. They are over 8 years old and their past history is well known as they have been used as experimental animals since calf-hood.

The agents responsible for the effect on appetite do not appear to be unsaturated contaminants in the paraffins. Both the odourless kerosene and odourless solvent are highly refined products, being 97% unsulphonatable. Fluorescent contaminants which may cause a rancid taste in medicinal liquid paraffin are mentioned by Sollmann (1948). The British Pharmacopoeia specifies that liquid paraffins shall not show fluorescence in daylight. None of the samples used here fluoresced in daylight, but under a Wood's filter ultra-violet source, both the odourless solvent and the odourless kerosene were found to fluoresce mildly. The fluorescing ingredient(s) however, seem unlikely to be involved, since Soltrol 170*, an odourless solvent of similar properties to Stanvac Odourless Solvent and containing 98.5% unsulphonatable material, does not fluoresce, although it also was found to have a marked effect on appetite (Fig. 4d). It seems probable, therefore, that the saturated hydrocarbons themselves are responsible.

The mechanism of the depression of appetite was not determined. The fact that bypassing the rumen did not prevent the depression, and the lack of effect on the morning of treatment in Exp. 1 and 4, indicate that any direct effect due to the taste of the material was only of minor importance.

There was no evidence of abnormality of the activity of the rumen musculature of cow 293 during Exp. 3, other than some minor irregularity of rhythms on the morning of treatment. However, recording from a single point—the dorsal rumen sac—is of only limited value in assessing the functioning of the whole organ.

The most likely explanation is irritation of the lower gut. Corrosive lesions have been described by Deichmann *et al.*, (1944) in poisoning from lighting kerosene, but the relative crudeness of this material compared with the highly refined paraffins used here precludes close comparison.

The Use of Liquid Paraffins for the Treatment and Prevention of Bloat

It might be expected that the very light liquid paraffins would mix through the rumen contents more readily and, therefore, relieve bloated cows more rapidly than the heavier paraffins or the vegetable oils. Reid and Johns (1957) found however, that these very low-viscosity oils were not superior to the heavy oils in effectiveness. Because

* A product of Phillips Petroleum Co., Bartlesville, Oklahoma. Sample supplied by Fruitgrowers Chemicals, Ltd.

of their deleterious effect on appetite, their use as bloat remedies does not appear justified.

In this country, kerosene is not infrequently given to animals for a variety of reasons, and even petrol has been used as an emergency drench for bloated cows. In Britain, deodorized kerosene is commonly used as a bloat remedy (Barker, 1954). It may be that, because of the delayed effect, any subsequent depression of the appetite has not been associated with dosing with very light paraffins. In testing such products for toxicity therefore, the intake of test animals should be observed over several days after dosing.

A reduced intake may well account for the "protection" from bloat for "several days" that is sometimes claimed for treatments. Certainly in Exp. 1, 2, and 3 the treatments given could be said to have provided protection. Since the loss of intake does not appear to be made up, this protection must be debited to production or to body reserves.

Of the paraffins used in this investigation, heavy liquid paraffin of medicinal grade seems to be the most acceptable. Only short term effects were considered here, but in the experiments reported in the next paper (McDowall *et al.*, 1957) heavy paraffin was given to lactating cows for several weeks without adversely affecting milk production. However, in using heavy paraffins over such periods, recognition must be given to the other risks inherent in the ingestion of mineral oil, that have been mentioned in the Introduction. Some aspects of these effects are dealt with in the above-mentioned papers.

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EFFECTS OF INGESTION OF PARAFFINS BY RUMINANTS

II. INGESTION OF HEAVY LIQUID PARAFFIN BY MILKING COWS IN RELATION TO YIELD AND COMPOSITION OF MILK AND TO PROPERTIES AND FAT-SOLUBLE VITAMINS OF BUTTERFAT

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Summary

Ingestion by milking cows of heavy liquid paraffin (B.P.) at the rate of 75 ml. twice daily for 26 days did not affect the yield of milk, the fat and solids-not-fat contents of the milk, or the iodine and saponification values of the butterfat. There was a continuous fall in the carotene content of the blood, reaching a maximum depression of 40% after about 16 days of treatment, and a 20% reduction in vitamin A ester content of the blood. Parallel with the blood changes, the carotene level of the butterfat fell by 40–50%, and the vitamin A level was slightly lowered. The vitamin A alcohol level of the blood was unaffected. Recovery of blood carotene to control levels was slow; it was almost complete 3 weeks after cessation of treatment. These results are interpreted as reflecting a reduction in carotene absorption caused by the ingestion of paraffin oil. Xanthophyll absorption appeared to be unaffected. Tocopherol absorption was reduced. After three weeks' treatment the level of tocopherol in the blood had fallen by 40% and in the butterfat by 20%.

The butter from cream obtained from treated cows was visibly lighter in colour than the control butter. It is concluded that, provided ingestion of mineral oil has no harmful effects on the health of the cow, administration of liquid paraffin could be used as a means of reducing the depth of colour of the butter. This reduction of colour, however, would be accompanied by a lowering of the vitamin A and vitamin E potencies.

Introduction

Use of vegetable oils as antifoaming agents for the treatment and prevention of bloat in dairy cattle has been recommended by Johns (1954), Reid (1955), and Reid and Johns (1957). From a study of the effects of various oils on the properties of milk, cream, and butterfat, McDowall *et al.* (1957) concluded that peanut oil was the most acceptable, because it had the least effect on the quality of these products.

Peanut oil, however, is relatively expensive. Liquid paraffins, known to be equally effective in bloat control, are cheaper. In the first paper of this series it was shown (Reid, 1957) that, of the grades of paraffin commercially available, medicinal paraffins of B.P. quality are the most suitable, because they have little effect on the feed intake of the animal. The present investigation was arranged to provide information on the effect (if any) of continued ingestion of the heavy grade of medicinal liquid paraffin by milking cows on the yield of milk and butterfat and on the properties of the butterfat.

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It is known that ingestion of mineral oil by animals and by humans affects the absorption of carotene and, to a lesser extent, of vitamin A.

Burrows and Farr (1927) and Dutcher *et al.* (1927) showed that rats were unable to utilize the vitamin A of butterfat when the latter was mixed with liquid petrolatum. Rowntree (1931) concluded that if the vitamin A of the diet was not adequate the addition of liquid petrolatum to the diet of rats would cause a vitamin A deficiency to appear. Jackson (1931) found a vitamin A deficiency in rats when butterfat and petrolatum were fed in mixed form but little decrease of vitamin A when they were given separately. Mitchell (1933) found that in the rat 10 to 12 times as much green spinach was required to furnish a unit of vitamin A when accompanied by 0.5 ml. of mineral oil, and about 8 times as much when the two were fed separately at times at least 6 hours apart. Paraffin oil with a higher melting point, and a wax mixture, were less detrimental than the fluid oil. Jackson (1931; 1934a) found that administration of mineral oil separately from restricted doses of yellow corn interfered considerably with the utilization of carotene from corn. The effect of mineral oil on assimilation of carotene was found to be greater than on assimilation of vitamin A. Dutcher *et al.* (1934) found that when butterfat was fed at low levels to rats in presence of mineral oil its effective vitamin A potency was lowered. They explained the harmful effect of the mineral oil as due to selective solution of the carotene into the mineral oil, with which it was excreted. They suggested that the hydrocarbons of the unassimilated mineral oil have a greater solvent effect on the hydrocarbon carotene than do lipids of the intestinal tract and that this difference prevents intestinal absorption of carotene in presence of mineral oil. Curtis and Horton (1940) found that rats utilize vitamin A equally well whether it is administered in mineral or cottonseed oil, but With (1940) found a 50% reduction in absorption of vitamin A when it was administered in a mineral instead of a saponifiable oil. The corresponding reduction in absorption of carotene was 80–98%. Burns *et al.* (1951) found that the amounts of mineral oil necessary to reduce dustiness in mineral mixtures and in alfalfa meal seriously diminished the utilization of β-carotene by rats. As little as 0.08% of mineral oil in the diet had a significant effect. The effect on vitamin A assimilation was not so great as that on carotene assimilation, but it was significant at the 0.16% level. Increasing the dietary fat level from 5% to 10% did not improve the utilization of vitamin A or carotene in the presence of 0.32% of mineral oil in the diet.

Similar effects of liquid paraffin on carotene and vitamin A assimilation in humans have been recorded. Curtis and Ballmer (1939) found that liquid petrolatum interfered with absorption of carotene from food, but that petrolatum saturated with carotene at 37°c. (0.28%) had no effect on carotene absorption. Curtis and Kline (1939) reported that feeding of crystalline carotene in vegetable oil caused a rise in blood carotene and that this rise did not occur when mineral oil was given at meal times along with the carotene. Alexander *et al.* (1947) found that continued ingestion of mineral oil in the form of salad dressing by 20 subjects on a normal unrestricted diet resulted in a moderate decrease in blood plasma carotene. They found the vitamin A level to be unchanged.

There are in the literature no reports of studies on the effect of regular administration of mineral oil to cows on milk yield and

composition, or on the general characteristics and vitamins A and E potency of the butterfat.

Experimental

Selection and Maintenance of Cows. For investigations on the characteristics and on the carotene and vitamin A contents of butterfat, monozygotic twins have been shown to have very marked advantages over unrelated cows (McDowall and Patchell, 1957). The properties of the butterfat from the cows of each twin pair are either almost identical or they show a consistent relationship to each other throughout the season, changing in a similar manner for the same change in environment.

In the present experiment, three pairs of these twins were used. The trial was carried out in the autumn. At this time the cows were in the later stages of lactation, i.e., conditions were such that even with monozygous twins some variations in milk yield and composition were to be expected. The cows were maintained out-of-doors on a ryegrass/white-clover/red-clover pasture.

Quantity of Paraffin Administered. For the control of bloat by spraying of bloat-inducing pastures with oil, dose rates of 90–180 ml. of oil per cow per day have been recommended (Reid 1955). In an alternative method of control, by covering the drinking water with a film of oil, a dose rate of 90–120 ml. per cow per day has been found necessary (Reid, unpublished). For the present experiment, therefore, a dose rate of 150 ml. (approximately 5 fluid ounces) was chosen, given as a drench of 75 ml. after each milking*. One cow of each twin pair (T26, T94, T98) was treated with mineral oil and the other was maintained on the same grazing area, but without treatment, as a control.

Treatment and Sampling Dates. The trial was divided into three periods:

1. Pre-experimental period: 21–25 March, 1956.
2. Experimental period: 25 March–20 April.
3. Post-experimental period: 20–27 April.

Sampling dates, except for tocopherol estimation, are shown in Figs. 1–8. Samplings of blood were continued for some time after the end of the post-experimental period for sampling of milk and butterfat (Figs. 6–8).

Data Recorded and Methods of Analysis. Milk yields were measured daily. Night-and-morning composite samples of milk were taken at 2–3-day intervals for estimation of fat and solids-not-fat contents. Samples of cream were similarly taken from which butterfat was isolated by churning (Dolby, 1954) for determination of butterfat characteristics and of carotene and vitamin A contents.

Iodine values were estimated by the Wijs method, and saponification values by the British Standard method (1938). Carotene and vitamin A in the butterfat were estimated according to the procedure outlined by McDowell (1949). In a number of samples the vitamin A in the butterfat was fractionated into alcohol and ester by the method of Thompson *et al.* (1949).

* The oil used was Stan-Vac white oil "890", kindly supplied by the Vacuum Oil Co. of N.Z. Ltd.

Blood samples taken at appropriate intervals during the period of the trial were assayed for content of carotenoids and vitamin A by the method of Thompson *et al.* (1949).

In some of the butterfat and blood samples tocopherol was estimated by the method shortly to be described by Worker (1957).

Results

The results obtained are given in Figs. 1 to 8. The lines in the upper three sections of the figures present the data for the individual cows of each pair and those in the bottom section give the means of the values for the three control and the three treated cows, respectively.

Milk Yield (Fig. 1), *Fat Content, Solids-Not-Fat Content, Iodine Value* (Fig. 2) *and Saponification Value* (Fig. 3). There is no evidence that these were significantly affected by the administration of heavy paraffin.

Carotene Content of the Butterfat (Fig. 4). Within a few days of commencement of administration of heavy paraffin the curves for the carotene content of the butterfats from the treated cows began to diverge from the corresponding curves for the control cows. The magnitude of this difference increased steadily during the first three weeks of the period of treatment. Thereafter it remained fairly steady. At the close of the period of treatment the carotene content of the butterfat from the treated cows was little more than half the corresponding values for the control cows. These differences persisted without appreciable change during seven days following cessation of drenching.

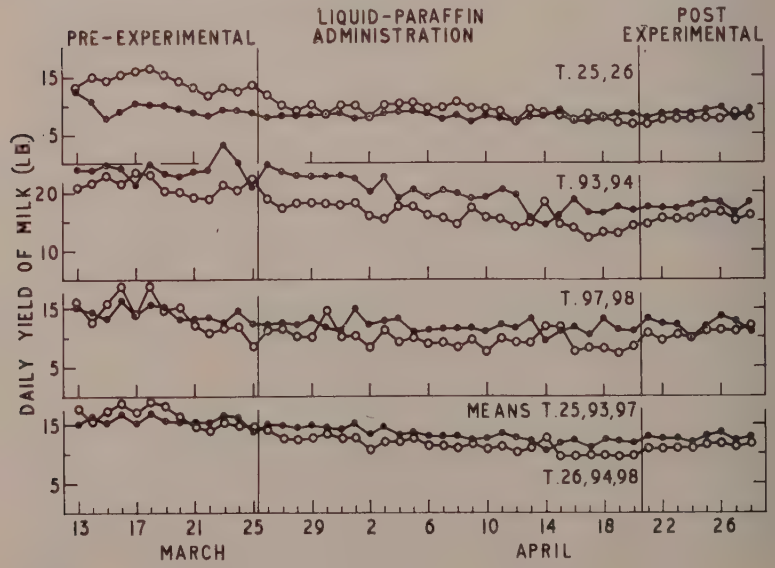

Fig. 1.—Daily yields of milk from control cows and from cows ingesting heavy paraffin.
— . — . — Control — o — o — Experimental

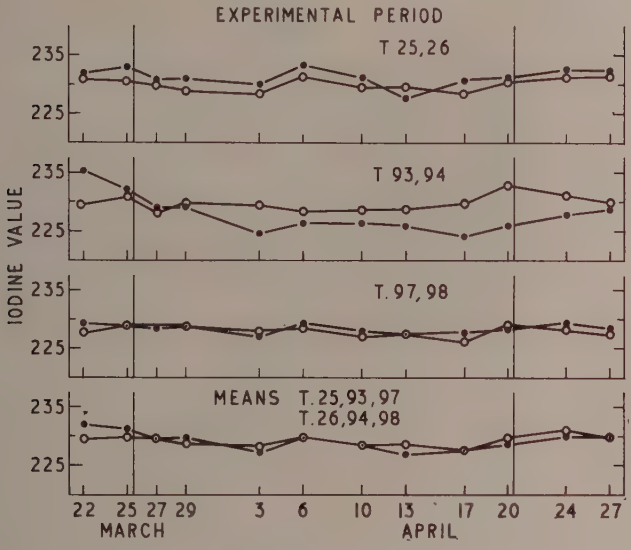

Fig. 2.—Iodine values of butterfat from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

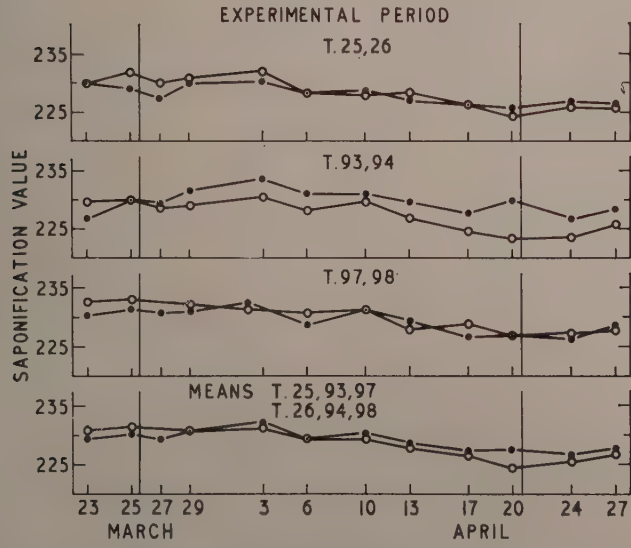

Fig 3.—Saponification values of butterfat from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

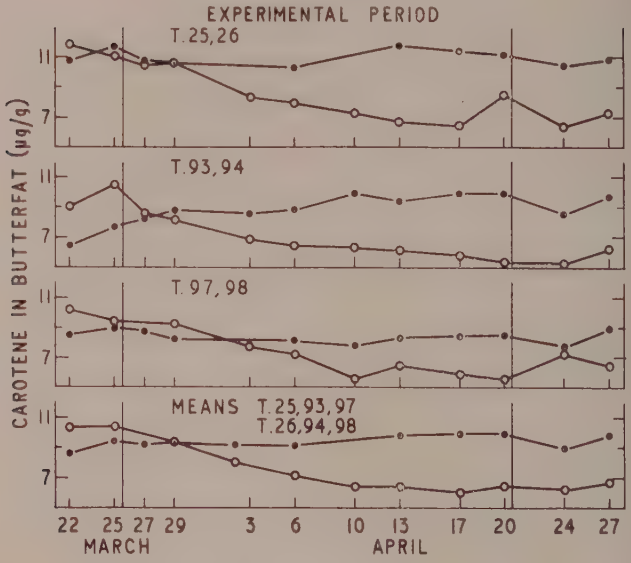

Fig. 4. Carotene contents of butterfats from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

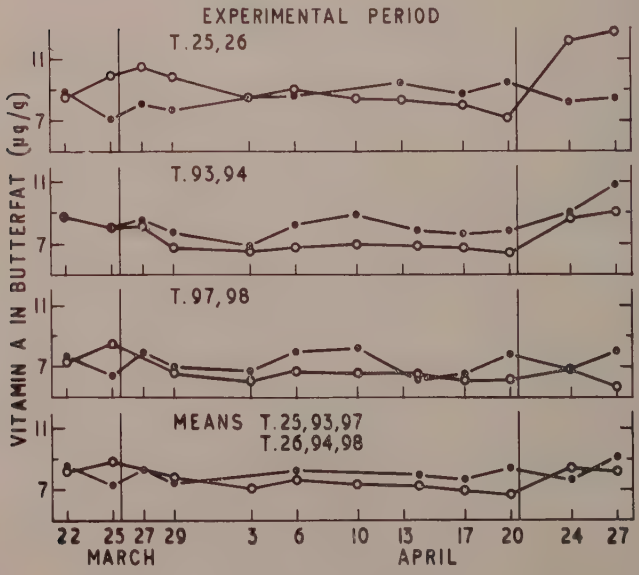

Fig. 5.—Vitamin A contents of butterfat from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

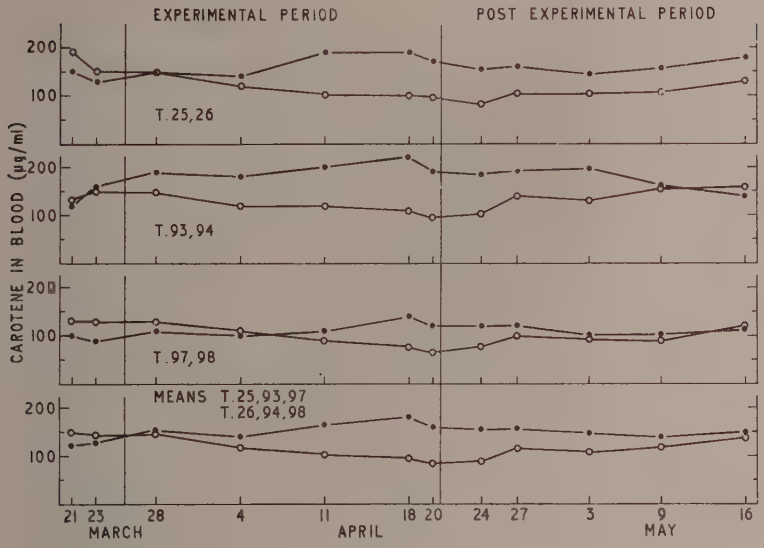

Fig. 6.—Carotene contents of blood from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

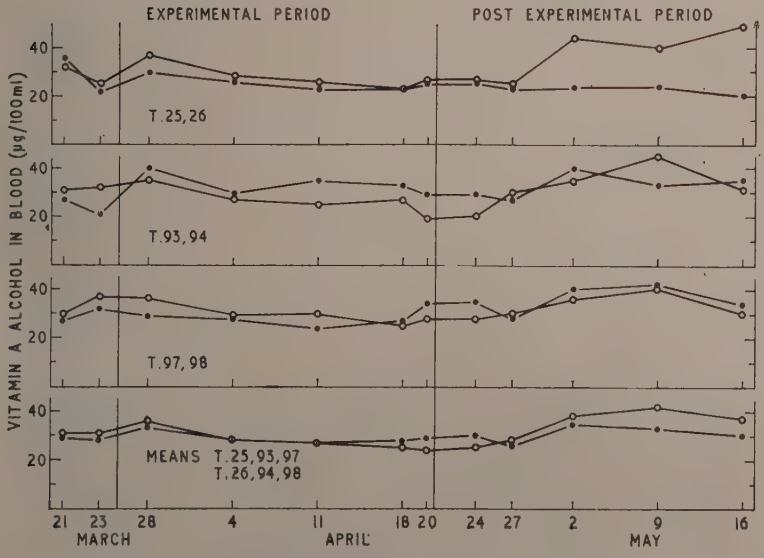

Fig. 7.—Vitamin A alcohol contents of blood from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

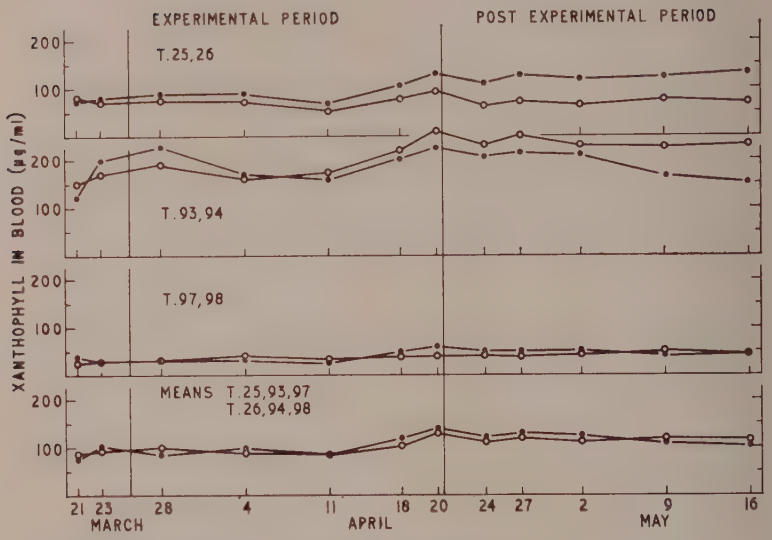

Fig. 8.—Xanthophyll contents of blood from control cows and from cows ingesting heavy paraffin.

— . — . — Control — o — o — Experimental

Since, unfortunately, sampling of the butterfat was discontinued at this stage, it is not possible to state for how long thereafter the differences persisted (see, however, section below, "Carotene Content of Blood").

Vitamin A Content of the Butterfat (Fig. 5). There is some evidence of a slight reduction in vitamin A content of the butterfat resulting from administration of heavy paraffin, but the effect was much smaller than for carotene. In all the samples examined the vitamin A alcohol content of the butterfat was uniformly low. The levels found represented only about 5% of the total vitamin A present; and there was no evidence of any change in these levels caused by ingestion of paraffin oil.

Carotene Content of the Blood (Figs. 6, 7, 8). There was a marked fall in the carotene content of the blood of the treated animals (Fig. 6), parallel with the fall in carotene content of the butterfat (Fig. 4). After cessation of administration of heavy paraffin the difference gradually diminished over a period of about 3 weeks. The parallelism between carotene content of the butterfat and carotene content of the blood during the period of administration of heavy paraffin and for the first seven days thereafter may be extrapolated to give the tentative conclusion that the carotene content of the butterfat will be affected for some 2–3 weeks after cessation of ingestion of heavy paraffin at the dose rate used, viz., 75 ml. twice daily.

Vitamin A Content of Blood. Vitamin A ester was estimated on three sets of blood samples taken on 20, 24, and 27 April, i.e., at the end of, and during the week immediately following, cessation of administration of the heavy paraffin. The average results, in μg/100 ml. plasma, were:

	20 April	*24 April*	*27 April*	*Average*
Control cows	2.1	2.8	2.4	2.4
Treated cows	1.6	2.3	1.9	1.9

There was thus a small but consistent difference in vitamin A ester content of the blood resulting from the treatment with heavy paraffin.

Xanthophyll Content of the Blood (Fig. 8). The xanthophyll content of the blood was not significantly altered by administration of heavy paraffin.

Tocopherol Contents of Blood and Butterfat. Samples for estimation of tocopherol content were taken on 21 March, i.e., at commencement of the pre-treatment control period, and on 18, 20 and 24 April, i.e. at and immediately after completion of the drenching period. The results are given in Table 1. At commencement of the

TABLE 1. Tocopherol Contents of Blood and Butterfat from Control and Treated Cows

Date	Cow No.						Means		
	25	26*	93	94*	97	98*	Control	Treated	Difference
				Tocopherol in Blood (μg/100 ml)					
21/3/56	500	520	510	490	430	540	480	487	+7
18/4/56	590	390	580	400	450	260	540	350	−190
20/4/56	560	330	550	380	400	220	503	310	−203
24/4/56	500	280	520	350	420	230	480	290	−190
				Tocopherol in Butterfat (μg/g)					
20/5/56	29	24	20	14	30	24	27	21	−6
24/5/56	—	—	19	17	28	22	24	20	−4
27/5/56	35	28	24	20	24	19	28	22	−6

*Paraffin-treated cows.

trial (21 March) the tocopherol contents of the bloods of the cows of each twin pair were comparatively uniform. After the drenching with heavy paraffin during 3–4 weeks, however, there was a marked and consistent difference, representing a 40% reduction in tocopherol content. This percentage change in tocopherol content of the blood is of the same order as the percentage change, noted above, in the carotene content of the blood.

The tocopherol level in the butterfat from the treated cows at the end of the treatment period, average 21 μg/g (Table 1), was consistently lower than that for the control cows, average 26 μg/g, but the proportional change was only 20% as compared with the 40% change for the blood tocopherol levels.

Discussion

The results show clearly that, with cows as with non-ruminants (see review of literature above), the ingestion of heavy liquid paraffin reduces the assimilation of carotene and of tocopherol from the food. In the present experiment, the reduction in carotene assimilation is evident as a 40% reduction in the carotene content of the blood, as a 20% reduction in vitamin A ester content of the blood, as a 40–50% reduction in the carotene content of the butterfat, and as a slight reduction in the vitamin A content of the butterfat. The effect on tocopherol absorption is shown by a 40% reduction in the blood level and a 20% reduction in the butterfat level of this vitamin.

The generally accepted explanation of the effect of paraffin oils on absorption of fat-soluble substances is that put forward by Dutcher and his co-workers (1927, 1934) for carotene. These authors (1934) consider that a proportion of the pro-vitamin in the digesta becomes dissolved in the liquid paraffin and is excreted with the latter in the faeces. Support for this explanation is given by the demonstration of an increased faecal excretion during mineral-oil dosing of rats (Dutcher *et al.* 1934) and of humans (Mahle and Patton, 1947); and by the finding of Curtis and Ballmer (1939) that paraffin previously saturated with carotene at body temperature is without effect on the assimilation of the pro-vitamin. The method whereby the paraffin takes up carotene from the digesta, and the site of this action, are, however, imperfectly understood.

It is claimed by Dutcher *et al.* (1934) that carotene, because of its hydrocarbon composition, is likely to be preferentially dissolved in the hydrocarbon paraffin. This is not completely valid as carotene is actually more soluble in tri-glyceride fats (such as arachis oil) than it is in heavy liquid paraffin. Glycerides and heavy paraffin however are miscible in all proportions. Hence in mixtures of the two a considerable amount of absorption of paraffin by the glyceride fats and *vice versa* can be expected. It may well be, therefore, that the transfer of carotene to the ingested paraffin may commence in the stomach by the solution of carotene-laden dietary fat in the mineral oil. The main site of transference is probably the small intestine.

The processes of fat digestion offer peculiar opportunities for the uptake of fat-soluble substances by paraffins. The first stage, emulsification of the fats, would seem to ensure an intimate mixing of lipid and mineral oil. The importance of such mixing is underlined by the work of Jackson (1931), Mitchell (1933) and Curtis and Kline (1939), showing that liquid paraffin has greater effect when it is given simultaneously with the food than when given separately from the food. Emulsification may start in the stomach, but mainly occurs in the small intestine, aided by the action of bile salts, and of fatty acids and glycerol mono-esters formed by partial hydrolysis on the fat globule surface (Frazer *et al.* 1944; Frazer and Sammons, 1945). The state of the liquid paraffin in the small intestine varies. When given to a starved rat the hydrocarbon remains only coarsely dispersed, ranging from particles of 10 μ diameter to large masses of free oil (Frazer *et al.*, 1944). In the non-starved animal, however, much of the hydrocarbon is emulsified (Alvarez, 1947). Whether or not emulsification occurs seems to depend on the presence of dissolved fat in the hydrocarbon. If the proportion of

dissolved fat reaches 50%, very fine emulsions are formed, and as much as 55–65% of the paraffin may then be absorbed through the gut wall (Daniel *et al.* 1951). Since, normally, in non-ruminants at least, only small amounts of paraffin are absorbed, the greater proportion of the hydrocarbon emulsion must usually be of a greater particle size than the maximum of 0.5 μ for absorption found by Frazer *et al.* (1944). Although there is no experimental evidence on this point, it seems possible that dissolved carotene in any mineral-oil emulsion absorbed would become available to the animal. It is not known whether the dissolved fat in unabsorbed paraffin-fat particles is hydrolysed in the same way as pure lipids, or whether the compound particle is excreted as such. Gage and Fish (1924) claim that butterfat in mixtures of butterfat and mineral oil is digested, leaving the paraffin as a residue. If this is so, it seems likely that the carotene will remain dissolved in the paraffin globule. Since in the present experiment there was no change in the iodine and saponification values of the butterfat during administration of paraffin oil, it would appear that the oil has no effect on the absorption of tri-glycerides.

In the second stage of fat digestion, tri-glycerides are hydrolysed to mixtures of di- and mono-glycerides, fatty acids, and glycerol. This process continues as the digesta move down the small intestine, while absorption is also taking place, so that the proportions of the various components change. Estimates of the extent of hydrolysis vary, but probably less than 50% of the tri-glycerides are split (see review by Frazer, 1952; and Bergstrom and Borgstrom, 1955). As tri-glyceride globules are broken up, dissolved fat-soluble substances such as carotene will be released and will be available for partitioning between lower glycerides, unsplit fat, and, if present, liquid paraffin. Partitioning will, of course, depend on the physical properties of the individual fat-soluble substances. Some information on the partition coefficients of carotenoids as between hydrocarbons and more-polar solvents is given by Patracek and Zechmeister (1956).

In the case of ruminants, several factors would seem to increase the opportunity for uptake of fat-soluble materials by ingested paraffin. Hydrolysis of glycerides in the rumen will cause an early release of dissolved carotene, etc. The reservoir effect of the rumen and the comparatively continuous outflow of digesta from that organ (Phillipson, 1952) favour mixing of fat and paraffin, although the rapidity with which oil seems to be lost from the rumen (Reid and Johns, 1957) will tend to counter these factors. The length of the small intestine (Dukes (1952) quotes figures of 46 metres for the ox, 26.2 metres for the sheep and goat) will also favour mixing and exchange. It should not be overlooked that the grazing dairy cow takes in daily some 450–700 g. of crude lipids of which approximately 10–15% (50–100 g.) is cuticular waxes and hydrocarbons. What part, if any, the latter play in the poor assimilation of carotene by ruminants (e.g., Hjarde *et al.* 1954) is not known, but it would be expected that they would have an effect similar to that of administration of paraffin oil.

Interference by mineral oil in the assimilation of vitamins A (Rowntree, 1931) and D (Dutcher *et al.*, 1927; Jackson, 1934b; Smith and Spector, 1940a, b), of tocopherol, and of vitamin K (Elliott *et al.* 1940; Anon, 1946) have been reported, and in all cases the general

mechanism is considered to be solution of the vitamin in the liquid paraffin.

Grazing ruminants, however, are entirely dependent on pasture carotene as a source of vitamin A. The conversion of carotene to vitamin A takes place in the intestinal wall (Kon *et al.* 1955), i.e., away from any possible contact with the unabsorbed paraffin oil remaining in the intestine. The changes in vitamin A levels found in the present experiment therefore reflect the changes in carotene absorption. Thus the blood level of vitamin A ester, the form in which the newly produced vitamin enters the circulation, was reduced by 20%, which in turn probably accounted for the fall in butterfat vitamin A level. Vitamin A alcohol levels in the blood were not affected by the treatment. This is according to expectations, since the alcohol is the form in which the vitamin A is released from the extensive liver reserves.

It is not clear why xanthophyll levels in the blood were not affected by paraffin treatment. Nor is it clear why, whereas both carotene and tocopherol levels in the blood were reduced by much the same amount —about 40% to 50%—butterfat carotene was reduced by 50%, but butterfat tocopherol by only 20%. The decrease in tocopherol levels is being further investigated.

Colour of Commercial Butter. The colour of the butter made from the creams from the treated cows during the process of isolation of butterfat for analysis was much lighter than that of butters from the control cows, parallel with the results given above for carotene content of the butterfats. To the butter-factory operator this finding that the colour intensity of butter can be lowered by administeration of paraffin is of particular interest. In countries such as New Zealand where cows are fed mainly on green pastures and where the herds are predominantly of the Jersey breed, butterfat has a high carotene content and the butter is therefore of a bright-yellow colour. Where also the cows are all calved down within a short period during the early spring the butterfat and the butter at this time have a still higher colour due to the high carotene content of early post-colostral fat. A recent survey has shown that the bright-yellow colour of butter from pasture-fed Jersey cows is greatly appreciated by a considerable section of the butter consumers in Great Britain; but other groups in Great Britain and a majority of consumers in Europe prefer a butter of paler colour—partly because they have become accustomed over the years to the use of butter of this type. If paraffin oil administration as a bloat preventative were to become generally adopted in the butter-producing areas, it would have the effect of markedly lowering the colour tone of the butter. This would mean a simultaneous reduction in vitamin A potency, although for butter produced from pasture-fed cows the reduction would not be critical. Alternatively, if butter of lower colour tone were required for a particular market it would be possible to obtain it from Jersey cows by administration of heavy liquid paraffin to the cows, or preferably, to draw the shipment from the supplies produced at the time when paraffin oil is being used for prophylaxis against bloat.

CONCLUSIONS

The results submitted show that ingestion of liquid paraffin by cows at dose rates used for bloat control markedly affects the assimilation of fat-soluble substances. Further work is required before the significance

of these changes and their possible sequelae can be assessed. Even if, as seems likely, they are not important for the non-pregnant animal, they may possibly have a basic importance for cows during pregnancy. Until the extent of the repercussions of paraffin ingestion has been more clearly defined, it seems prudent to hold in abeyance any recommendations for paraffin administration for prevention and control of bloat in lactating cows or for colour reduction in butter.

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BLOAT IN CATTLE ON RED CLOVER

II. THE TYMPANOGRAPH, A SIMPLE APPARATUS FOR RECORDING ABDOMINAL GIRTH CHANGES IN STALLED ANIMALS

By C. S. W. Reid, Plant Chemistry Laboratory, Department of Scientific and Industrial Research, Palmerston North

Summary

The corrugated-rubber-tube stethograph has been adapted to record abdominal girth changes in stalled cattle.

The tracings not only record the primary girth changes due to feeding or bloating, but also reflect activities such as respiration, feeding, body movements, urination, defecation, and, under favourable conditions, rumen movements and belching.

Examples of the uses of the technique are given.

Introduction

In the investigations described in Part I of this series (Johns, 1954) rumen distension was assessed by eye and by palpation of the left flank. Subjective measurements of this type have serious limitations for quantitative experimentation, and the present paper describes a simple apparatus—an adaptation of the corrugated-rubber-tube stethograph—for the continuous recording of abdominal-girth changes in stalled cattle. Use is made of the fact that changes in the length of a corrugated rubber tube due to stretching cause directly proportional changes in its internal volume.

Description of the Apparatus

The Sensitive Element

The corrugated tubing used for most recordings was the hose from the standard British army respirator, care being taken to select a firm specimen since soft tubes may collapse on extension. Those used here were of black rubber without a cloth covering. A satisfactory alternative is B.O.C. Antistatic Anaesthetic hose.*

The ends of the corrugated tube are closed with rubber bungs (Fig. 1a) over which they are tightly clamped by means of worm hose clips. The remaining attachments to the element are shown in the figure.

The performance of the respirator hose elements is improved by introducing a compression force, using strands of elastic rubber stretched internally between the ends (see Fig. 1a). This reduces the permanent extension so that the element is operated through a range of less error. The rubber must be stretched only lightly so that it will neither limit extension of the element, nor cause an undue increase in the force required to extend it. By this means the elements used were shortened by about 4 cm., and the non-linearity error of 10% for 15 cm. extension reduced by a half.

Medium-weight stethoscope tubing was used for the lead connecting the element to the recorder, although any flexible, non-kinking tubing of

* Manufactured by the British Oxygen Company, London.

approximately 3 mm. bore is suitable. The lead is connected to the recorder through a three-way tap.

The Harness

The element is held in position on the animal by means of a harness of heavy brass chandelier chain. This material has the advantages that it is light, inextensible, and non-corroding, and lies flat on the animal. It has the minor defect of being rather soft. The harness consists of two lengths of this chain, one end of each being permanently attached to the ring on the element. These chains pass around the animal's

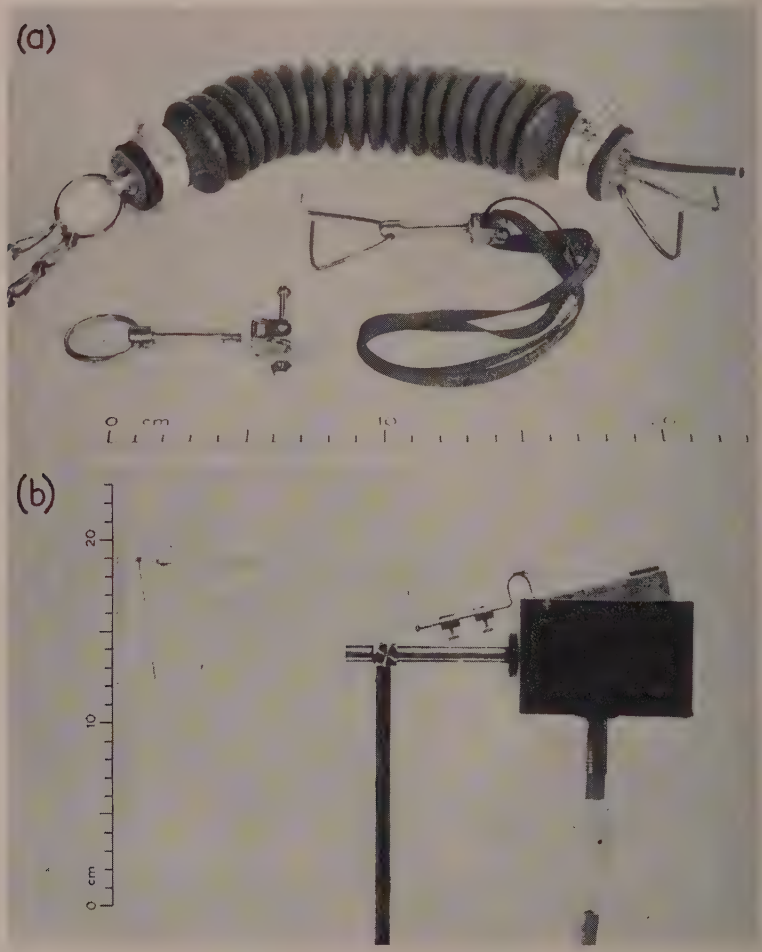

Fig. 1(a).—A sensitive element made from respirator hose. Note wire hooks for adjusting chain harness. In foreground are a pair of anchor studs, showing the stirrups for the rubber strands that are stretched inside the element. (b) A modified float recorder. Note the use of various features of the float to impart rigidity to the lever which works in the reverse direction to normal. Smaller counterweights than usual are required.

body, one anteriorly, one posteriorly, and then fit over the hooks at the opposite end of the element (see Fig. 1a and 2). The front chain, approximately 4.5 m. long, is looped twice around the chest to avoid slipping, the anterior loop lying close behind the foreleg on the left hand side. The rear chain loops only once around the abdomen, passing in front of the right iliac crest, but otherwise lying as far back as it will go on the right hand side. The element itself provides the tension in the harness and any adjustment required is made by taking up or slackening off the chains on the hooks.

The Volume Recorder

The capacity of the volume recorder must be sufficient to cope with the full extension of the corrugated tube. With the present elements, this corresponds to a volume change of approximately 80 ml. A large tambour (7.5-cm. bowl) may be used, but a float recorder of standard pattern* is preferable.

Fig. 2.—General view of apparatus showing sensitive element in place, lead from element to recorder (inside window), signal key on top of feed box and wire connecting this to signal on kymograph. Note excess chain lying alongside element.

Since the volume changes in the recorder are the opposite of those occurring in the element, a first-order lever (made of 20-gauge aluminium wire) is used to invert the displacement of the recorder. The relationship

* Manufactured by Messrs. C. F. Palmer Ltd., London.

between the tracing and the time base is preserved by the use of a frontal writing point. To minimize the error introduced by the latter, the lever is tilted up at an angle of about 10° from the level of the float top. Details of the construction of the lever used with the float recorder are shown in Fig. 1b.

The Record

The tracing is made on smoked paper on a kymograph. A paper width of 19 cm. has been found most convenient as it allows room for simultaneous recording of additional information. A key beside the feed box and connected to a solenoid-operated marker, is used to signal the commencement of feeding, and also—by a code of pips—belches, urinations, defecations, and other movements. One-minute intervals are registered by a suitable time marker.

Operation

Setting up. The element is adjusted to its correct position, lying along a line running downwards and backwards across the left lumbar triangle, and midway between the rear rib and the left iliac crest, the hooked end resting against the transverse processes. By slightly stretching the element, just enough tension is given to the harness to keep the element in place and in reasonable contact with the flank of the animal. However, if the flank is sunken, good contact may be impossible at the start. The chains should be checked to see that they follow the shortest possible lines to avoid slipping.

Sufficient air is introduced into the system through the side arm of the three-way tap to bring the writing point close to the lower limit of its excursion. The tap is closed, and is not touched for the remainder of the recording, otherwise the relationship of the tracing to the starting point will be altered.

Recording. After recording commences, a short time is allowed before feeding the animal, to establish a steady base line.

When the writing point is close to the upper limit of its excursion, the chains are taken off the hooks, the element allowed to contract, and the chains replaced over the hooks in their new position. Such adjustment will be necessary two or three times during the course of bloating and again during deflation, when the procedure is reversed (see Fig. 4b). These adjustments will cause the element to move away from and return to the lateral processes.

Errors

There are five main sources of error in recording major girth changes by this technique:

(a) Apparatus errors. Calibration curves, obtained by plotting the displacement of the writing point against extension of the corrugated tube, are shown in Fig. 3 for various combinations of recorders and elements. With the "compressed" respirator hose and the float recorder, the deviation from linearity of 15 cm. extension of the element is about −5% This error is cumulative during a phase of girth increase involving adjustment to the element; it is self-cancelling during subsequent deflation.

(b) Varying contact between the rumen and the left flank and between the left flank and the corrugated tube.

(c) Components introduced from other parts of the animal's body by the chain harness.

(d) Variations in the placing of the corrugated tube on the flank.

(e) The constricting effect of the element and harness.

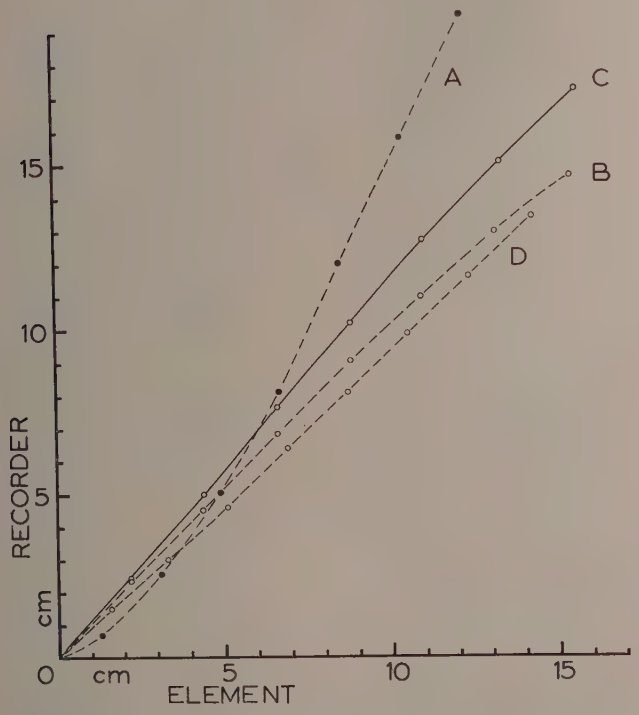

Fig. 3.—Calibration curves for different corrugated tubes and volume recorders (extension of element against displacement of writing point). A—respirator hose and large tambour. B—respirator hose and float recorder. C—"compressed" respirator hose (i.e. with internal rubber strands) and float recorder, the combination normally used. D—anaesthetic hose and float recorder.

In practice, these errors are minimized or standardized by operating the apparatus within its range of least error, and by standardizing the positions of the element and harness. The care with which these precautions are taken will largely determine the reproducibility of the records, the major remaining variable being the state of the animal at the commencement of recording.

RESULTS

Typical records are shown in Fig. 4. The major girth changes are best presented by plotting the altitude of the tracing against time, taking the altitude at the commencement of recording as zero. For the

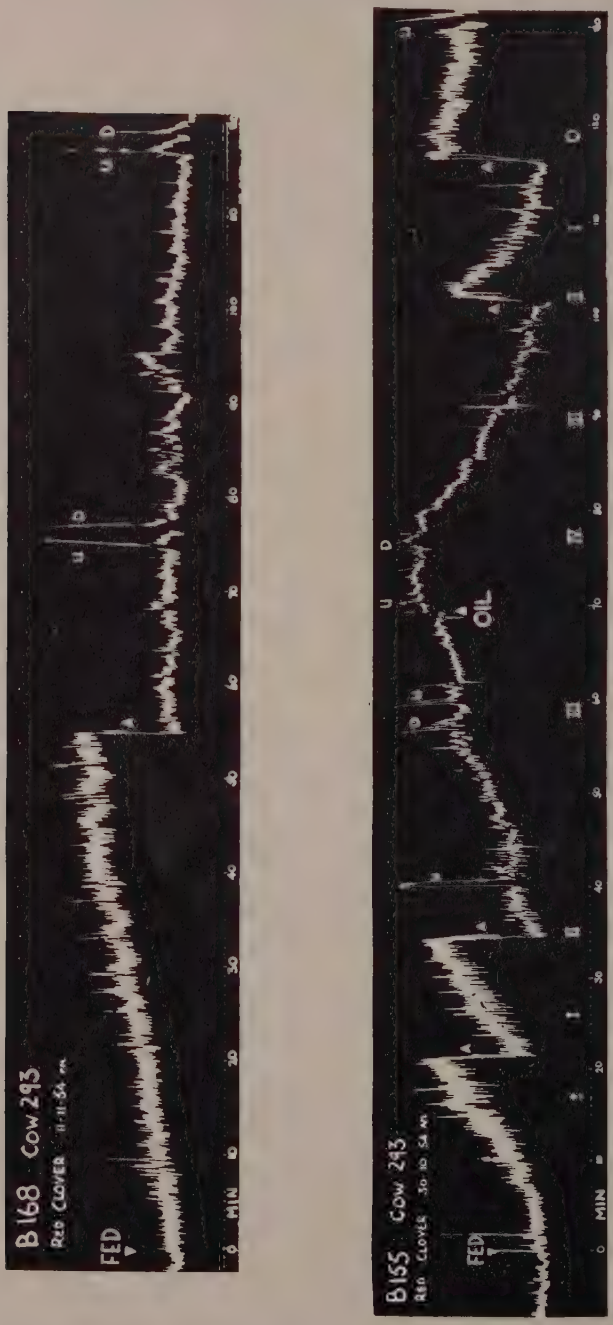

Fig. 4.—Typical tympanograph records of (a) feeding without bloating, and (b) feeding followed by bloating and treatment (a drench of 100 ml. of whale oil). A—adjustment of element on animal; D—defecation; U—urination; I–IV—bloat gradings (described by Johns, 1954). Cow 293.

curves shown in Fig. 5a, average heights (in inches) have been plotted at 10 min. intervals, allowance having been made for transient excursions due to activities such as urination, and for adjustments of the element on the animal.

The characteristic shape and form of the tracings are determined by three main types of activity:

(a) activity of or in the forestomach, including filling, bloating, deflating, rumen movements, and belches,

(b) respiration, coughs, and grunts,

(c) body movements associated with feeding, urination, defecation, restlessness, etc.

Changes in the Forestomach

The major changes in girth are due to filling, bloating, and deflating.

Filling. For a short period after feeding commences, there is often only a small change in the general altitude of the tracing. This is probably due to taking up of the "slack" in the animal's body, and the establishment of good contact between the body wall and the corrugated tube. As filling continues, in the absence of bloat, a steady increase in girth takes place (see Fig. 4a). The maximum extent of this increase varies. It is usually small when the animal is fed green grass, grass hay, or non-bloating clover hay. However, following the ingestion of green clovers, girth increases range up to the levels seen in the earliest stages of clinical bloat (curve A, Fig. 5a). When feeding stops, there is often a minor reduction in girth after which the girth usually remains about the same until about two to three hours after the feed was given, when a gradual decline commences.

When filling takes place in the presence of an anti-foaming agent, e.g., peanut oil, the girth change is very much reduced, resulting in comparatively flat tracings and curves (see curve D, Fig. 5a).

Bloating. When bloating occurs, the rate of girth increase is more rapid and the extent of the increase much greater than when filling takes place in the absence of bloat. A typical tracing from a bloating cow is shown in Fig. 4b and a curve derived from such a tracing in Fig 5a (B). If severe bloat occurs, there is usually a constant rate of increase up to grade III, followed by a period of decreasing rate and finally little change when grade IV is reached. At this stage, the skin is probably limiting further expansion.

The extent of the girth change for a given grade of bloat (as determined by palpation, Johns, 1954) varies both between animals and from day to day in the same animal. Between-animal variations are probably due to differences in the elasticity of the abdominal wall in different animals. The principal cause of intra-animal variation is the state of fill of the rumen at the commencement of recording resulting in a varying base line. Although no allowance is made for the latter error, the average girth increase is directly proportional to the bloat grading in any individual animal. This is illustrated in Fig. 5b which has been constructed from measurements of 34 tracings, chosen at random out of 140 made during two seasons from cow 294. In any

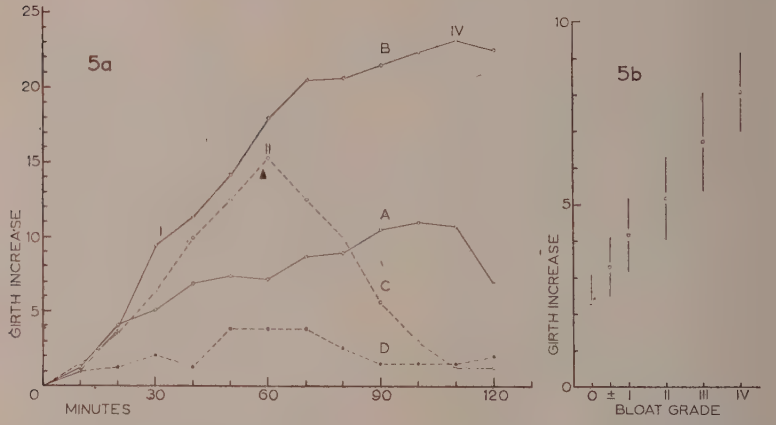

Fig. 5(a).—Typical girth change curves for cow 294 feeding on red clover. A—No bloat. B—Bloating to grade IV. C—Drenched with 50 ml. of raw linseed oil when moderately bloated (△). D—After a pre-feeding drench of 250 ml. of peanut oil. Girth change is measured as the change in altitude (in in.) of the tracing relative to its altitude at the commencement of feeding. (b) Correlation of girth increase with bloat grading system. Cow 294. Mean girth increase and standard deviation range plotted for each bloat grade. The grades are spaced along the X axis as if the steps between them are equidistant, with the exception of ± (see text).

one tracing, bloat gradings at points of equal altitude on the ascending and descending portions of the curve correspond closely.

Following effective treatments, deflation curves are usually sigmoidal, with the length of the initial period of slow or negligible girth change varying considerably.

Rumen movements and belching. Under favourable conditions, regular patterns due to ruminal contraction waves and to belching may be seen in the tracings (Fig. 6, 7). Good contact between rumen, flank, and element is necessary, and obscuring body movements must be at a minimum. Consequently the patterns are best recorded from well filled or bloated animals that have stopped feeding.

The patterns due to rumen movements alone, i.e., not accompanied by belching, are often ill-defined. However, records from moderately bloated (Grade II or III) animals strongly suggest the presence of vigorous rumen activity at this stage (Fig. 6b). Records from severely bloated (Grade IV) animals have always shown evidence of rhythmic activity (Fig. 6d) although interpretation of the patterns is often difficult.

Belching is associated with characteristic patterns (Fig. 6, 7) and is consequently easier to detect than rumen movements alone. The "belch" pattern was first recognized by stationing at the animal's head an observer who signalled belches by means of the key. Eructation was found to be always associated with these patterns (when conditions were favourable for the latter to be seen) but occasionally in a bloated animal a belch pattern occurred without an audible belch. This was confirmed later when a sensitive throat microphone and audio-amplifier were used to detect the belch sounds. Belch patterns were usually

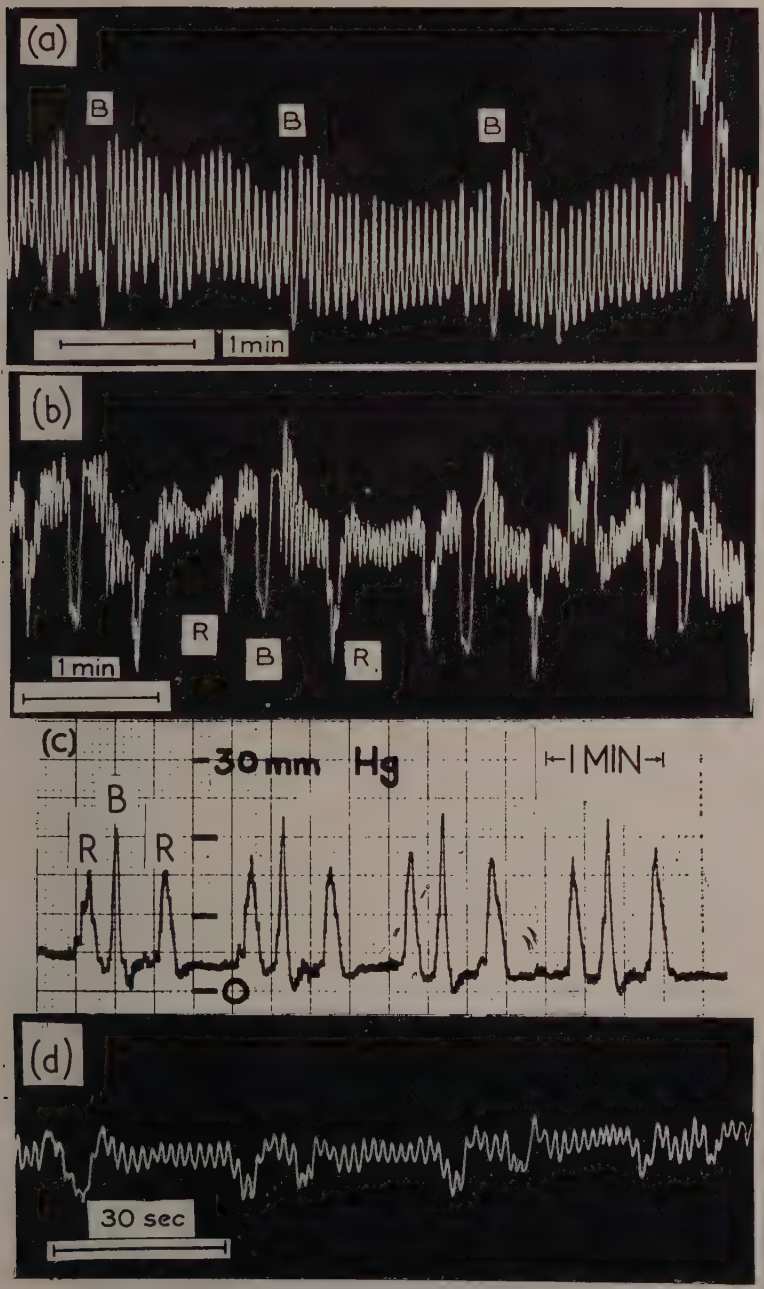

Fig. 6.—Examples of patterns due to rumen activity (R) and belching (B) in records from cow 294 (a) after feeding but not bloated, (b) moderately bloated and (d) severely bloated. In (a), belching may be distinguished but other activity is only suggested. Rumen activity and belching are clearly seen in (b) while (d) shows the persistence of rhythmic activity in severe red clover bloat in this animal. Note differences in the extent of respiratory excursions. Tracing (c) is a record of pressure changes in the dorsal rumen (not bloated) after 294 had been fistulated. Contractions which raise the rumen pressure cause a diminution of girth so that record (c) is the inverse of (b). The rhythm and time relationships are closely comparable in the two records. (all full size).

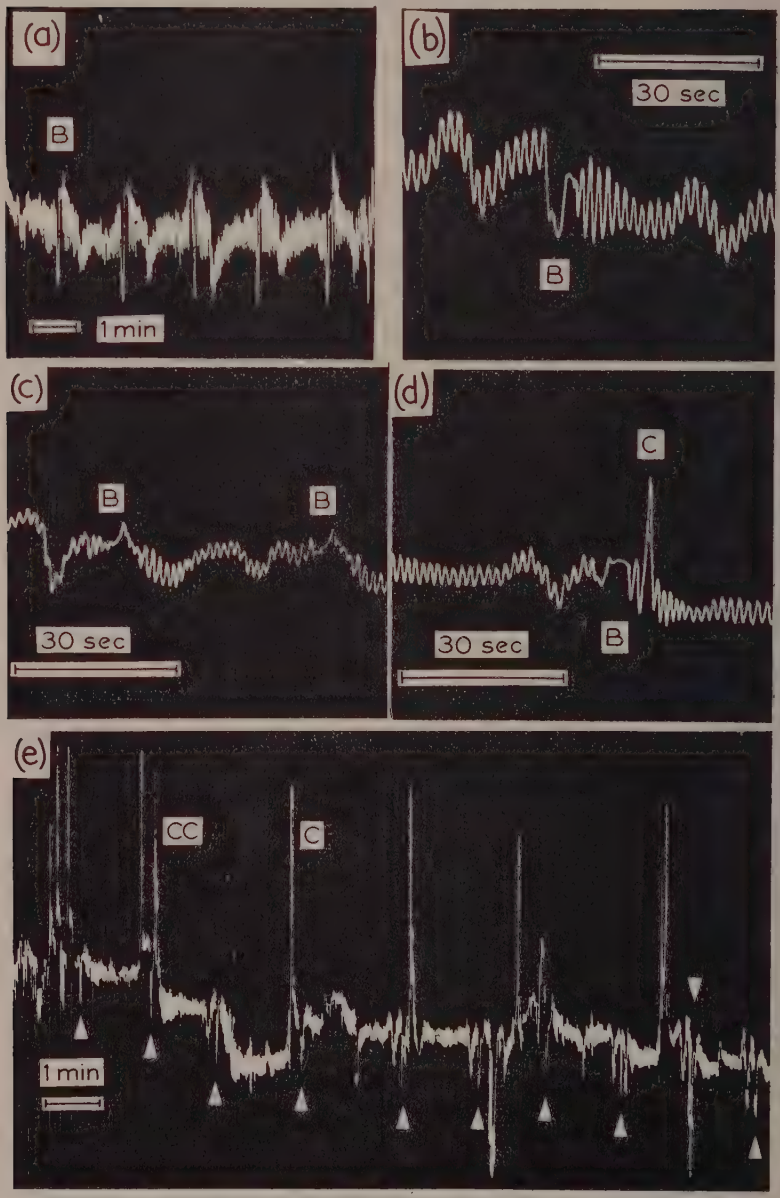

Fig. 7.—Typical belch patterns (B) from records of cow 293 after feeding, not bloated (a), and of 294 when mildly bloated (b) and when moderately severely bloated (c, d). The reduced respiratory excursions during belching and their temporary increase in size afterwards are clearly shown. Note the variation in the size of the downward belch spike, prominent in (a) and (b), almost absent in (c) and (d). In (d) there was a slight cough (C) after each belch. Coughing after a turpentine drench (cow 294, moderately bloated) is shown in (e). Note that coughs produce upward spikes whereas belches (arrows) are associated with downward spikes in this record. (All full size).

Fig. 8.—Step-like pattern of deflation corresponding to a series of belches (arrows) following a drench of crude whale oil (not shown). A—adjustment of element on animal. Cow 293.

prominent up to a bloat grading of III. Thereafter, they tended to become less clearly depicted and belching might become irregular with periods of up to 5 min. between. Typical belch patterns were always obtained during deflation following the administration of an anti-foaming agent such as peanut oil. On occasions the altitude of the tracing fell distinctly with each belch, producing a step-like tracing (Fig. 8).

Respiration

In tracings from bloating animals the amplitude of the respiratory excursions decreases steadily, and in severe bloat, is quite small (cf. Fig. 6a, b, and d). This is in part due to the overall change in the depth of respiration, and in part to the decreasing amount of abdominal respiration possible in the bloating animal. The amplitude increases again with deflation, which may be followed by a period of deep breathing.

The rate of respiration increases slightly with feeding in the normal animal. In bloated animals, the rate may be further slightly increased.

Miscellaneous

The tracings also record body movements associated with active feeding, head movements, urination, defecation, and the like. Again, after some experience, many of these movements can be detected directly from the tracings.

Preliminary trials have shown that satisfactory records may be obtained from sheep. It is important to keep body movements to a minimum with this subject, since they have a relatively greater effect than in the case of the cow. Rumen activity was well recorded.

Satisfactory records have also been obtained from the right flank of fistulated cows. As would be expected, the tracings so obtained are not directly comparable with those made from the left flank. However, they are useful and allow simultaneous recording of other phenomena to be carried out through the fistula.

DISCUSSION

In quantitative investigations of tympanites, a method of assessing the degree of bloat is needed that is objective, reproducible, and sufficiently sensitive to detect small changes; its value will be the greater if continuous, permanent recording is possible. No technique is yet known which satisfies all these requirements.

The most obvious external sign of bloat is the distension of the flanks caused by the abnormal intra-ruminal pressure. Because of the large variation between animals and the fact that expansion is limited, although pressure may continue to increase, simple visual inspection allows only gross estimation of the state of the animal. More reliable information is provided by manual palpation of the left flank to judge the internal pressure. With practice, when used in conjunction with other signs and with a knowledge of the idiosyncrasies of individual animals, this method allows a reproducible grading of sufficient accuracy for many routine purposes. It is, however, a subjective technique, and it often fails if fine discriminations are attempted. Grading by palpation of animals with rumen fistulae is most difficult.

To improve upon manual palpation, Kleiber (1945) attempted to measure the intra-ruminal pressure through the flank by means of the "tympanometer". Some workers claim that with sufficient practice, reproducible results may be obtained with this instrument, but others consider that the "operator error" is too high and that too little account is taken of the variables introduced by the properties of the body wall of the animal (R. W. Dougherty, A. T. Phillipson, personal communications). However, even if direct methods were used to measure the pressure, it is not at all certain that the figure obtained would necessarily indicate the state of the animal. Although the intra-ruminal pressure must be of primary importance in the earliest stages of bloat, it is not known what secondary effects it may have which may be more important in causing distress or death. The cerebro-spinal fluid pressure or the oxygen and perhaps the carbon dioxide concentrations in the blood, for example, may provide far more significant indices of the higher grades

of bloat. Moreover, the pressure required to produce signs of distress would appear to vary widely from animal to animal, and even in the same animal, from day to day (Dougherty *et al.*, 1955).

Because of the limitations of girth change as an index of bloat, the tympanograph does not offer greater accuracy than previous methods. Indeed, to offset the effect of variations in fill at the start of recording, reference points must be obtained by other methods, such as palpation. The major advantages that the tympanograph does offer are objective information in the form of a continuous, permanent record, and a sensitivity that allows easy detection of trends. It records indirectly phenomena that may be used in assessing distress—respiration, urination, defecation, restlessness, lowing, etc.—and it can provide some information about rumen activity and belching. For these reasons, it has proved invaluable in the studies reported in this series of papers. It has already been used in four types of experiment:

(a) Following the day-to-day variation in the potency of feed,

(b) Evaluating different treatments (this is dealt with in detail in Part III, Reid and Johns, 1957),

(c) Comparing bloat caused by the ingestion of different feeds,

(d) Examining the pathogenesis of red-clover bloat.

In the latter connection, tympanograph tracings have suggested the presence of foam in the rumen of the normal animal. Tracings made when animals, starved overnight, were given anti-foaming agents before the morning feed, often showed moderate decrease in girth, suggesting the presence of foam in the rumen *before* feeding. Marked decreases in girth were found in similar experiments in which these agents were given to normal cows after feeding to satiety on red clover. This would appear to indicate that foam formation is responsible for a large part of the apparent fullness of the unbloated animal after the ingestion of clover. Both these deductions were confirmed later when animals with rumen fistulae became available, and give support to the idea that, initially at least, bloat is a matter of degree. Tympanograph tracings also give striking evidence of the rapidity with which bloating may take place. In such instances, even though the animal may have been starved overnight, the girth increase is virtually linear from the start of feeding, through the stage of the first detectable signs of bloat (approximately 20 min.), to the severest stage (approximately 40–45 min.). The absence of any inflexion in such curves, other than that due to the normal taking up of "slack" in the first 5 min., would suggest that either the state of the rumen contents before feeding is an important factor or, that if the plant contributes essential materials, they are being released very rapidly in the rumen.

The presence of well-defined patterns, due to rumen movements and belching, in tracings from moderately bloated cows strongly suggests that paralysis of the rumen plays little part in the initiation of the condition. Even in severe bloat, when no motion of the flank may be visible, rhythmic activity has always been recorded. No deductions as to the quality or effectiveness of this activity can be made from tympanograph tracings.

The form of the "belch" patterns provides some evidence regarding the source of the synchronous pressure rise throughout the rumen and

reticulum that accompanies belching. Dougherty *et al.* (1955) noted, during the beginning of the eructation reflex, a transient rise in intrapleural pressure. Coughs and other events associated with a rise of pressure at that point tend to increase the girth and are recorded as upward spikes in these records (see Fig. 7d, e). Upward fluctuations occur in the belch patterns, but are usually preceded by *downward* spikes (see Fig. 6a, b, 7a, b). The latter differ from girth changes due to rumen movements without belches in that the respiration excursions are simultaneously much reduced, suggesting that an active contraction of the abdominal wall is taking place. As would be expected, the size of the downward spikes tends to diminish with increasing degree of bloat, and they may be almost absent in severe bloat.

An investigation of rumen activity in bloating cows, using direct methods, is in progress.

ACKNOWLEDGEMENTS

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SEASONAL AVAILABILITY OF FOOD AND ITS INFLUENCE ON THE LOCAL ABUNDANCE OF SPECIES OF BUMBLE BEES IN THE SOUTH ISLAND OF NEW ZEALAND

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(*Received for publication, 15 April 1957*)

Summary

Food supply can determine the population density of bumble bees and moreover can determine the species composition in a district by being available when one species and not when another first emerges from hibernation and at nest founding.

Copious early spring food and dearth of late spring food favours *B. terrestris,* whereas the reverse favours *B. ruderatus.* Adequate food supply throughout the whole of spring enables *B. ruderatus* to compete favourably with *B. terrestris* and sometimes to outnumber it, e.g. in the Havelock-Okaramio district.

New Zealand prior to European settlement was almost completely bush clad save for two relatively small areas of open country that were tussock grassland. The settlers as they cleared the land sowed European pasture plants of which red clover was one, but since there were no native insects capable of effecting pollination of this plant it failed to set seed. Hive bees, introduced in 1839 and soon well established throughout the country, only rarely contributed to the profitable production of red-clover seed, so bumble bees were introduced for this purpose. In 1885, after several previous unsuccessful attempts to establish bumble bees, success was obtained with *Bombus terrestris* (L.) and *B. ruderatus* (Fab.). In subsequent years other importations were made from which *B. subterraneus* (L.) subsp. *latreillellus* (Kirby) and *B. hortorum* (L.) became established.

Today, *B. subterraneus* and *B. hortorum* appear to be confined to the southern half of the South Island. *B. subterraneus* is local and rare but *B. hortorum* is evenly distributed throughout its range and relatively numerous. *B. terrestris* and *B. ruderatus,* however, occur throughout the country but vary considerably in their distribution and relative numbers from district to district.

The factors that limit the range and numbers of *B. subterraneus* and *B. hortorum* have not been investigated but observations made during a general survey of the entomological factors affecting seed set in red clover in New Zealand show how seasonal availability of food may influence the distribution and numbers of *B. terrestris* and *B. ruderatus.*

After the queen emerges from hibernation in early spring the food reserves within her body are soon depleted. The developing eggs must be nourished, and considerable energy is expended in searching for a suitable nesting site and establishing the nest. Then the queen has to provide for and tend the first brood. This period of nest founding is the critical one and unless there is an abundant supply of nectar and pollen the efforts of the queen are doomed to failure. At this time of the year relatively few plants are in flower.

* The information presented in this note was gained while the author was a member of the staff of the Entomology Division, D.S.I.R., Nelson, N.Z.

Once nests are founded and the queens are relieved of foraging duties, then the availability of nectar will be reflected in the size of the individual nests, which, even if reduced through scarcity of food, will still contribute to the maintenance of the species through eventual but limited queen production.

B. terrestris in the time since its introduction to New Zealand has adapted itself to the milder climate and has only an incipient hibernation period. A few queens can be seen foraging on almost any sunny day throughout the winter and indeed at times the whole colony overwinters (Cumber (1949) one instance, and personal observations made in this survey, six instances). The main emergence, however, in Nelson and Marlborough takes place in August, reaching its peak about the end of August, and most nests are founded by the end of September. *B. ruderatus* however still hibernates completely and does not emerge until mid September or early October and it is November before nests are founded. A similar situation was found to occur in Canterbury by McBurney (1941) and in Manawatu by Cumber (1954).

If the foregoing hibernation factors are considered in relation to the seasonal availability of nectar and pollen, it is clear that an abundant early food supply will establish *B. terrestris* successfully to the detriment of the later-emerging *B. ruderatus* queens, which can be expected to compete only if food is plentiful. Honey bees where present will also be increasing in numbers as the spring advances, and will further increase the competition for food. Seasonal availability of food can thus be expected to determine not only the local adundance of a species, but also its presence or absence.

In addition to the question of competition for food, there is also that of nesting sites. The requirements of the two species appear to be similar. A population of *B. terrestris* launched by a copious early spring food supply will have first choice of nesting sites and be producing an increasing worker population by the time *B. ruderatus* queens emerge from hibernation. Insufficient nest sites therefore accentuates the benefit to *B. terrestris* of a copious early spring food supply and its limiting effect on the numbers of *B. ruderatus,* especially if there is a dearth of food at the time when the latter species is nest founding.

A study made of two areas within 25 miles of one another, one Grassmere, where *B. terrestris* predominates, and the other Havelock-Okaramio district, where *B. ruderatus* predominates, illustrates these points. At intervals between September 1953 and November 1954 visits were made to these areas and the population, composition, and foraging behaviour of the bumble bees present was noted.

Grassmere is one of the driest districts in New Zealand; it is classified by Garnier (1951) as dry, subhumid, with a moisture index of −20 to 0. The country is mainly rolling tussock-covered hills with the few flat areas in the valleys in cultivated pasture. Here, then, the only forage for bees in the spring is supplied by the homestead gardens, the willows around the creeks, and the exceedingly sparse exotic weeds on the roadsides and in the pastures. In one particular property (G. Avery, Grassmere Road), in the homestead block there was a hillside of about four acres planted intermittently with tree lucerne (*Cytisus*

proliferus L.)*. The present survey revealed that this is by far the most important early flowering plant, both as a source of nectar and pollen, more especially in the drier districts, e.g. Grassmere, where it was almost the only source of early spring food.

This plant starts flowering towards the end of May and continues in flower until late September, during the whole of which time it is a reliable source of nectar and pollen. It is worked by *B. terrestris,* often to the exclusion of all other plants. These trees supported a large number of queens. When the tree lucerne stopped flowering, there remained only the flowers in the home garden and some horehound (*Marrubium vulgare* L.) and nettle (*Urtica urens* L.) around the sheep yards to provide food for the bumble bees. This meant then a considerable reduction in available nectar and pollen. From the end of September until the flowering of the early summer roadside weeds in November, food was scarce. During this time *B. ruderatus* emerged from hibernation to face a scarcity of food accentuated by intense competition from the large population of *B. terrestris* which moreover was daily being swelled by the workers emerging from the newly established nests. It is likely therefore that few *B. ruderatus* queens manage to establish nests, for in the previous season on this property not a single *B. ruderatus* was seen working the lucerne (*Medicago sativa* L.) crops. *B. terrestris* greatly outnumbers *B. ruderatus* throughout the district and tree lucerne, which is commonly grown here, undoubtedly contributes to this situation.

Havelock-Okaramio, on the other hand, is a relatively wet district; it is classified by Garnier (1951) as perhumid with a moisture index of 100 and above. It comprises a series of valleys between hills clad with beech (*Nothofagus* spp.). The beech is attacked by a coccid (*Coelostomidia assimile* (Mask.)) which for the greater part of the year produces honey-dew, and bumble bees readily use this for food. In addition, from mid-October onwards many flowering weeds i.e. periwinkle (*Vinca major* L.) and volunteer clovers, both *Trifolium repens* L. and *T. pratense* L., growing on the hillsides and bush edge ensure an adequate food supply for bumble bees. From early spring onwards, therefore, there is no dearth of food, thus when *B. ruderatus* emerges from hibernation and is nest founding there is no competition for food with *B. terrestris.* Under these conditions *B. ruderatus* is able to compete with and outnumber *B. terrestris.* Dumbleton (1948) considered that *B. ruderatus* constituted 80% of the population in this district. Evidently scarcity of late spring food (in October and November) in addition to a copious supply of early spring food is a necessary condition for the dominance of *B. terrestris.*

Other districts in the South Island have not been so consistently sampled but both McBurney (1941) and Dumbleton (1948) found *B. terrestris* to be dominant on the Canterbury Plains and the present survey showed that *B. ruderatus* predominated in the foothills of mid Canterbury at Fairlie, and in the hills surrounding the Taieri Plains,

* It has been shown (Gurr, L. (1955) *N.Z. J. Sci. Tech. A37:* 300), that *B. terrestris* is the most efficient bumble-bee pollinator of lucerne (*Medicago sativa* L.) and Grassmere has particularly high yields of lucerne seed. Climatic conditions here are very favourable for lucerne seed production but insect pollinators are essential before seed will set, and it is claimed that were it not for the predominantly *B. terrestris* population of bumble bees made possible by the plantings of tree lucerne the yields would be considerably lower.

Otago. Garnier (1951) classifies Canterbury Plains as moist sub-humid, moisture index 0 to 20, and Fairlie and Taieri Plains as humid, moisture index between 20 and 100. Climate and consequently vegetation on the Canterbury Plains approximates to that found at Grassmere except that all the land here is under cultivation and with short-rotation pastures even fewer weeds are available to provide a food source for bumble bees. The homestead gardens and roadside weeds, therefore, are the only food source during the early spring; this enables *B. terrestris* to predominate as it does at Grassmere. Climatic conditions at Fairlie and Taieri are similar to those at Havelock-Okaramio but with a slightly lower rainfall and consequently the beech forest does not occur and no honey-dew is available. On the rough untilled ground of the hills, weeds are plentiful, however, and these provide a continuous supply of food throughout spring and early summer and *B. ruderatus* is thus able to predominate as it does at Havelock-Okaramio.

These comparisons support the findings made in the two areas studied in Marlborough, and are supported by the red-clover seed yields which can be taken as a reliable index of the district pollinator force—in New Zealand, bumble bees. Further, since the long-tongued *B. ruderatus* is a more efficient pollinator of red clover than the short-tongued *B. terrestris* (Brian, 1954) red-clover seed yield is also an index of abundance of *B. ruderatus*. Okaramio, Kaituna, and Havelock, all in Marlborough, and 88 Valley in Nelson, have very high red-clover seed yields, up to 800 lb. per acre, and Fairlie and Taieri, although they have appreciably lower yields than this, still have much higher yields than those of the Canterbury Plains.

It is concluded, therefore, since variation in dominance of *B. terrestris* and *B. ruderatus* coincides with variations in trophic conditions, that food is the determining factor. Moreover, the variation in seasonal availability of food in conjunction with the difference in hibernation behaviour of the two species is the means by which it operates.

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STIMULATION OF WOOL GROWTH BY THYROXINE IMPLANTATION

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Summary

The results from recent field trials on the effects of l-thyroxine when implanted in the sheep are discussed.

Particular attention has been directed towards the effect of this hormone on the wool produced.

The evidence from all trials demonstrates that an increased rate of growth of wool varying from 5 to 15% of total fleece weight was obtained.

The increased weight of wool produced is partly due to additional length of staple, there being no apparent change in "count". This results in an improvement in the commercial grade of the whole fleece and reduction in the percentage of fleeces with cotts and breaks; at the same time there is no difference in yield.

No adverse effects on either mortality rate or the fertility of mature ewes have been observed.

Introduction

During the course of some fundamental studies on the responses of sheep to photoperiodic stimuli it was shown that these treatments had a profound effect on rate of growth of wool (Hart, 1953). This discovery has led to considerable speculation as to (a) the method of reception of the stimuli and (b) the physiological mechanism by which the growth of wool is influenced. Subsequent experiments seemed to indicate that the pituitary body was implicated (Hart, 1955). This being so, the natural sequence was to test whether the responses in growth of wool could be evoked through the use of hormone therapy. One of the several hormones tested, l-thyroxine, produced an encouraging response when injected in the form of an aqueous solution (Hart, 1954). Later work was directed towards determining the optimum level of dosage and the best method of administration, and this appeared to be in the vicinity of 60–90 mg. of l-thyroxine administered in the form of a pellet implanted under the skin during the midsummer-autumn period of the year (Hart, 1955).

In view of the interest aroused in the possible use of l-thyroxine in this manner as a means for stimulating wool growth, it has been decided to present the results obtained from field trials carried out in 1955 and 1956 before elaborating further the abridged accounts previously mentioned.

The trials were planned to be conducted in both the North and South Islands of New Zealand so as to obtain results from animals farmed under different climatic conditions and systems of management. The aim in all trials was not to interfere with the normal farm management on the particular farm in the district selected, and thus obtain results under conditions as natural as possible.

Experimental Methods

Site of implantation on the animals was the same in all trials, at a point behind the shoulder, about two inches down the side from the

centre line of the back. A short-needled implanting gun was thrust downwards parallel to the ribs and immediately underneath the skin so that the pellets were ejected to lie subcutaneously in the connective tissue, and under no circumstances in muscle tissue.

The date of implantation varied slightly for each trial but was in general during February or March, when the rate of growth of wool on sheep under natural conditions is around its peak (Coop, 1953). The dates of implantation for individual trials are shown in Table 1.

TABLE 1.

Trial	Date of Implantation	Locality	Age & Breed of Sheep	Condition of Sheep at Implantation	Feed Conditions
A	18.2.55	Banks Peninsula	5-year-old Border Leicester × Halfbred	Good	Excellent
B	29.2.56	Canterbury Agric. College	Mixed-aged stud Romney	Medium	Medium
C	8.2.56	Banks Peninsula	Mixed-aged Romneys	Medium	Poor
D(1)	2.3.56	Canterbury Agric. College	Mixed-aged Corriedales	Medium	Medium-Poor
D(2)	2.3.56	Canterbury Agric. College	Mixed-aged Romneys	Medium	Medium-Poor
E	16.2.56	Waikato	Mixed-aged Romneys	Medium	Very poor

The animals were ewes, and basically producers of "crossbred" type of wool in all trials except one, where the fine-woolled Corriedales were used. They were mixed-aged flocks and included two-tooths in all except trial A. The condition of the ewes at the time of implantation was assessed on the basis that "medium" represented sheep in good store condition, which were able to maintain themselves with slight improvement when under "medium" feed conditions (Table 1).

In trial E, owing to the threat of facial eczema, the sheep had to be shut up at the rate of 400 to the acre from 5 February until 26 April. During this period the average loss of live weight in the controls was approximately 10 lb., and in the implanted group 18 lb.

In all trials the ewes were randomized into their groups on the basis of age and of previous fleece weight, wherever this information was available. They were then identified as groups by paint branding or ear tags, or both, and they were run as one mob until shearing. Only ewes that weaned lambs have been considered in this analysis.

The dose was 90 mg. of l-thyroxine given in three 30-mg. pellets, except in trial D where a dose of 60 mg., in two 30-mg. pellets was also tested to enable a comparison to be made in the same flock between the two levels of implantation.

Wool measurements commenced at shearing, when each individual fleece, minus bellies and floor sweepings, was weighed and commercial "count" and grade assessed by a qualified wool classer from the Lincoln College Wool Department. A small sample was drawn from the midside of each fleece and these were bulked for yield test.

Fleece grading was based on descriptions published by Duncan (1944) and each of the seven described grades was further divided into two. The highest of these grades received 14 points and each succeeding grade 1 point less, so that a point's difference of 1.0 is equivalent to a difference of half a commercial grade.

RESULTS

The results obtained from these trials are shown in the following tables.

TABLE 2. GREASY WEIGHT, YIELD, AND CLEAN SCOURED WEIGHT

Trial	Treatment	No. of Ewes	Greasy Fleece (lb.)	Increase		Yield % (16% Regain)	Clean Fleece (lb.)	Increase	
				(lb.)	(%)			(lb.)	(%)
A	Control	47	8.5			65.4	5.6		
	90 mg.	42	9.6	1.1	12.9	66.0	6.3	0.7	12.5
B	Control	70	7.1			77.5	5.5		
	90 mg.	67	7.7	0.6	8.5	78.2	6.0	0.5	9.1
C	Control	426	9.4			71.8	6.7		
	90 mg.	87	10.0	0.6	6.4	72.4	7.2	0.5	7.5
D 1.	Control	92	6.6			68.4	4.5		
	90 mg.	66	7.0	0.3	4.5	66.1	4.6	0.1	2.2
	60 mg.	78	7.0	0.3	4.5	67.0	4.7	0.2	4.4
D 2.	Control	120	6.6			74.0	4.9		
	90 mg.	85	7.5	0.9	13.6	73.9	5.5	0.6	12.2
	60 mg.	82	7.6	1.0	15.2	72.4	5.5	0.6	12.2
E	Control	140	6.7			71.7	4.8		
	90 mg.	121	7.0	0.3	4.5	72.7	5.0	0.2	4.2

DISCUSSION

In spite of considerable variations in the nutritional level, which included one extreme case of starvation treatment in order to prevent facial eczema, wool production increased in all the treated groups. This was not accompanied by any increase in mortality or other measurable defect in sheep fed normally. Unfortunately the complete results on mortality were not obtainable from Trial E; there was however an indication that the mortality of the thyroxine-treated group was higher whilst they were on the severe sub-maintenance level of nurition in this trial. Nevertheless it is apparent that sheep fed at maintenance or above may safely be treated without any fear of illness or loss.

These trials have shown therefore that under normal farm conditions, without the provision of any additional supplementary feed,

TABLE 3. GRADE, COUNT, LENGTH AND PERCENTAGE BREAKS, COTTS, AND MORTALITY

Trial	Treatment	No. of Ewes	Average Grade	Increase	Average Count		Average Staple Length (cm.)	Breaks (%)	Cotts (%)	Ewe Mortality (%)
					Coded †	Bradford				
A	Control	47	8.4		8.7	48/50	*	59.5	*	3
	90 mg.	42	9.5	1.1	9.2	48/50	*	35.7	*	4
B	Control	70	9.8		8.3	48	12.4	3.7	0	0
	90 mg.	67	10.1	0.3	7.6	48	12.8	3.6	0	0
C	Control	426	8.3		9.2	48/50	17.0	11.0	23.9	1.7
	90 mg.	87	9.1	0.8	9.1	48/50	17.6	7.1	14.9	1.5
D 1.	Control	92	9.1		13.8	56	8.7	20.6	10.8	9.5
	90 mg.	66	9.5	0.4	13.6	56	9.3	15.9	3.4	5.5
	60 mg.	78	9.7	0.6	13.7	56	9.9			8.0
D 2.	Control	120	8.4		8.3	48	11.6	23.4	13.3	9.5
	90 mg.	85	9.4	1.0	8.3	48	13.4	22.7	8.4	5.5
	60 mg.	82	8.9	0.5	7.6	48	13.1			8.0
E	Control	140	7.3		9.5	50	13.5	44.6	11.4	*
	90 mg.	121	8.2	0.9	9.1	48/50	14.3	26.6	11.6	*

† 2 Coded intervals = 1 full Bradford count interval

* Records incomplete

it is possible that up to a pound of extra wool may be grown. In addition, the commercial grade of the whole fleece may be improved by as much as half a commercial grade in the case of Romney and crossbred types of wool.

Owing to the large numbers and extensive nature of these trials it was not possible to record the changes in live weights of the animals concerned. However, previous experience with housed animals had shown that the treated sheep will lose weight. The amount lost varies according to dose rate and nutrition; a 90-mg. dose may remove up to 8 or 10 lb. live weight from a ewe weighing between 110 and 120 lb. The weight loss commences about 5 to 6 days following implantation and usually reaches a maximum between the 14th and 21st days, after which it may remain static for a period followed by a gradual recovery. Two-tooths take longer to recover than older ewes and their weight loss is more apparent to the eye (Hart, unpublished data).

It is noteworthy that in Trial D where Romney and Corriedale ewes were compared under the same environmental conditions, the coarse-woolled Romney has given a significantly greater response than the fine-woolled Corriedale.

The same trial also shows that in the Romney there has been no reduction in the magnitude of the response obtained from the smaller 60-mg. dose compared with the larger 90-mg. dose.

That the increased weight of wool produced by the treated sheep is pure wool is shown by the yield tests, in which no significant differences were found between the thyroxine-treated and control sheep. Associated

with the increase in weight is the increased staple length of the treated sheep which varied from an average increase of 4 mm. in trial B, to 18 mm. in trial D2. There was no difference in fibre diameter as assessed visually according to the Bradford count system, but there was a definite reduction in the number of fleeces showing break or cotting in favour of the thyroxine-treated sheep.

It might be suggested that since it is generally regarded as not possible to get something for nothing, and since a precise physiological explanation for the response cannot yet be advanced, the sheep may suffer in some way. In this connection it should be pointed out that the animal does in fact undergo what might be regarded as complementary changes in that it loses a not inconsiderable amount of live weight within twenty-one days of implantation (Hart, unpublished data). Certainly this loss is gradually made good during the subsequent months, but nonetheless important changes have been taking place during this period in the animal. It could be argued that two things have been taking place in the animal which may be related in one way, and diametrically opposed in another. In the first instance the animals may be producing more wool at the expense of live weight. This could be due to an increased metabolic rate or a temporarily increased efficiency in the utilization of the products of metabolism or a combination of both. Another suggestion might be that an increase in the rate of mitosis of the wool-follicle cells had been induced and all that has occurred is a redistribution of the blood food materials to the areas of increased activity. This intrepretation seems a likely one in view of the findings of Ferguson *et al.* (1956a), that the thyroxine requirements in lambs for wool growth and secondary-follicle maturation are greater than those for general body growth. The second point is that the loss of live weight is a reversible process whereas the additional wool grown cannot be removed; so that if the wool has been grown at the expense of live weight then it is there permanently and when the regain of live weight takes place subsequently it does not do this by altering the amount of wool already produced, nor does it reduce the rate of growth of the wool during that

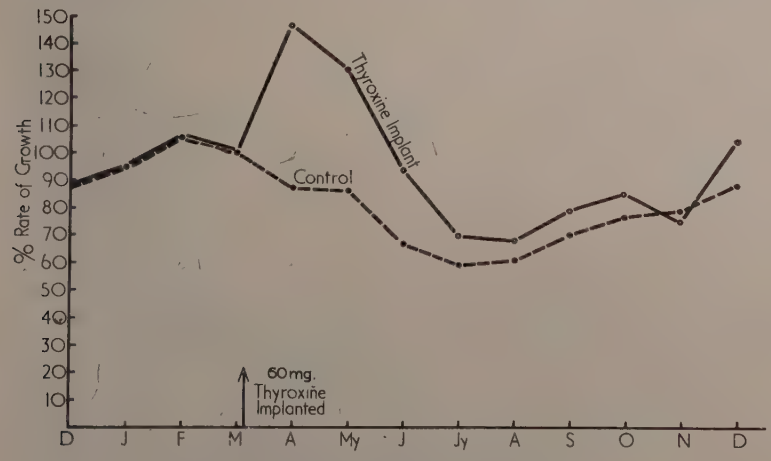

Fig. 1.—Effect of l-thyroxine on wool growth.

period. Fig. 1, reproducing the wool growth response curve from the 60 mg. implants of Hart (1955) clearly shows that during the remaining period of the year when the weight is being regained, the rate of wool growth from thyroxine-treated sheep does not fall significantly below that of the control.

Ferguson *et al.* (1956b) in Australia have stated that the extra wool grown as the result of what they term lower dose levels of thyroxine was offset by an increase in food consumption and loss of body weight so that the efficiency of wool growth was less in thyroxine-treated sheep than in the controls. This is contrary to the results obtained in pen trials by Hart (1955) when the treated animals had no additional feed whatever, but it may well be true for Merino sheep under Australian conditions since in the present trials Corriedales were less responsive than Romneys.

In any case, under the present New Zealand pastoral systems of sheep husbandry there is usually a surplus of feed available in late spring and early summer which is often above the ewe's requirements for maintenance and lactation. This results in the ewes unavoidably putting on surplus fat during the latter stages of lactation with the result that they are over-fat by weaning time. This situation is sometimes difficult to remedy before tupping season, even when the ewes are deliberately starved, and in some cases may be aggravated by continued growth of pasture in summer. Overstocking to reduce surplus fat is not always good for the ewe or the pasture, so it may prove possible by the use of thyroxine to provide a means of utilizing this excess of fat and feed at this time of the year particularly as the feed becomes available at the period of the year when the rate of wool growth is at its maximum (Coop, 1953) and response from thyroxine will be greatest. Indeed, in many fat-lamb-producing areas of New Zealand more often than not the use of thyroxine as a means of removing surplus weight prior to tupping may be justified on this score alone, and any improvement in wool growth or grade could be regarded as being an extremely welcome additional return.

It has been suggested by Ferguson that there might be some reduction in the economic life of the animal resulting from the long-term use of thyroxine. To date there has been no indication of this in the performance of cast-for-age ewes which have been given thyroxine and compared with ewes of the same age without thyroxine. The results in ewes receiving annual implantations from the two-tooth stage onwards have not yet been determined.

Generally, the economic life is determined by teeth (Barnicoat, 1957) and not physiological age, and thyroxine would have to influence tooth attrition in order to be of practical significance. So far as New Zealand is concerned, if there were a reduction of as much as, say, twenty per cent in the physiological lifetime of the animal it would still not bring it down to the present economic life now being determined by the teeth. Nevertheless, this point cannot be overlooked and trials have been commenced with young sheep whose lifetime production is still being studied.

The effect on fertility is also of extreme importance and a considerable amount of investigational work has already been completed on

this aspect. Pending the final completion of this particular section of the trials, it can safely be said that there has certainly been no reduction in the fertility of the mature ewes used in these experiments wherever normal managerial practices have been followed.

ACKNOWLEDGEMENTS

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EFFECTS OF INGESTION OF PARAFFINS BY RUMINANTS

III. EFFECT OF PARAFFIN OIL ON THE LEVELS OF CAROTENE AND VITAMIN A IN THE BLOOD PLASMA AND MILK FAT OF COWS—A FURTHER INVESTIGATION

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(*Received for publication, 1 May 1957*)

Summary

The effect of different amounts of orally administered paraffin oil on the levels of carotenoids and vitamin A in the blood plasma, butterfat, and subcutaneous fat of cows has been further investigated.

The depression of carotene and vitamin A ester levels in the butterfat and of carotene levels in the blood plasma has been shown to be proportional to the paraffin-oil dosage level and some evidence is presented regarding the mode of action of the oil.

The possible use of paraffin oil as a means of reducing the yellow colour of butterfat is discussed.

Introduction

In a previous communication (McDowall *et al.*, 1957) it has been shown that the drenching of cows with paraffin oil causes a marked depression in the carotene content of the butterfat and hence a reduction in the yellow colour of the butter produced from that fat. The intense yellow colour of New Zealand butter, particularly during the spring months, is frequently stated to be a factor prejudicing its sale overseas. If this is correct, any practical method of reducing the colour could be of considerable economic value. Paraffin oil has been shown to be effective in the control of bloat (Reid and Johns, in press) and, apart from its effect on the carotene, and to some extent the vitamin A, content of butterfat, does not appear to influence milk or fat yield or fat composition. It is one of the least expensive oils useful in bloat control and since bloat is most prevalent at the time when the carotene content of the fat is high, its wider use in bloat control would be not only of some immediate economic attractiveness but would also effect the desired reduction in the yellow colour of our spring butter without any extra inconvenience or cost to the industry.

Further work was therefore undertaken to investigate more fully the effect of oral dosing with paraffin oil on the levels of carotenoids and vitamin A in the butterfat, in the blood plasma, and in the body fat. In the main these experiments were carried out during the early spring when the carotene content of the butterfat was at its highest level and hence the butter most yellow.

Experimental

The experiments to determine the effect of different dosage levels of paraffin oil* were carried out during August, September, and early October, 1956. The cows used were from the herd of The Dairy

* The oil used throughout the investiagtion was a heavy medicinal grade "Ondina 33" as supplied by the Shell Co. of N.Z. Ltd.

Research Institute (N.Z.). They were all predominantly Jersey and were period and from the corresponding position on the other flank at the grazed with the main herd on typical dairy pastures throughout the experiments. In Exp. I, two animals were used and were each dosed with 300 ml. paraffin oil daily for a period of 22 days. The oil was administered as two equal drenches after the morning and evening milkings. In Exp. II, three cows were used and were each dosed with a different amount of oil for a period of 20 days. In this experiment the oil was mixed with a proprietary meal† (one part of oil to four parts meal by weight) and the mixture was fed to the animals, after the evening milking only, at a rate equivalent to an oil intake of 30, 60, and 120 g. oil per cow per day. Throughout Exp. I and II, blood samples were taken at intervals from the treated cows and from two untreated control animals. All milk samples were taken at the evening milking. In Exp. I the effect of paraffin oil on body fat was also investigated, subcutaneous fat samples being taken from the two cows. The fat samples were taken under local anaesthesia from one flank during the pre-experimental end of the period of paraffin oil administration.

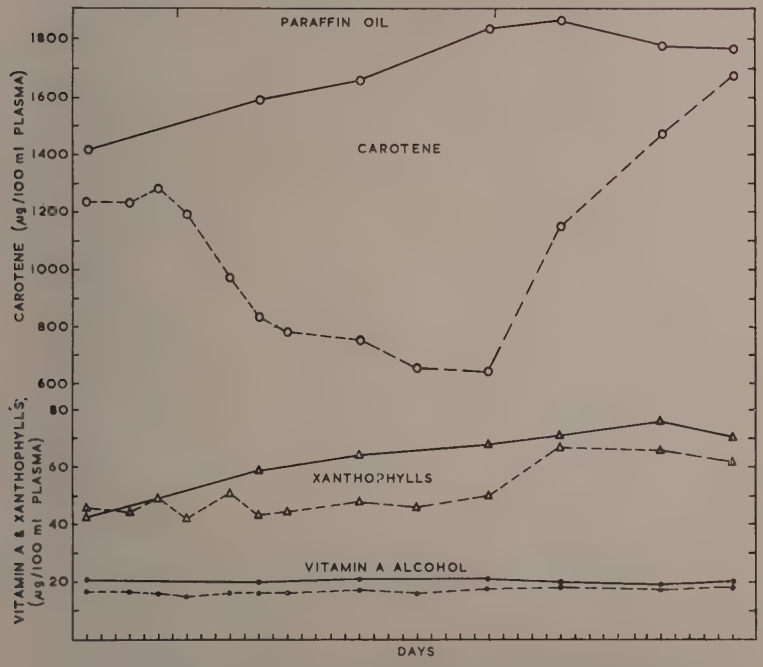

Fig. 1.—Effect of 300 ml. paraffin oil daily on the levels of carotenoids and vitamin A in the blood of cows. Control ———; treated - - - -; Carotene o — o; xanthophylls Δ — Δ; vitamin A alcohol . — .

In Exp. III, carried out in February 1957, two cows were used. One was dosed with 225 ml. paraffin oil daily for 20 days while the other

† This was compounded locally for feeding to milking cows and consisted largely of crushed barley.

received the same quantity of paraffin oil previously saturated with carotene at 37°C. (a mixture of natural and synthetic β-carotene, both supplied by L. Light & Co. Ltd.). In both cases the oil was administered as two equal drenches after the morning and evening milkings. In Exp. IV one cow was given a single drench of 2 g. vitamin A as an oily concentrate containing 160 mg. vitamin A per gramme of oil. Another cow received the same amount of vitamin A mixed with 225 ml. paraffin oil, also as a single drench. Milk and blood samples were taken at intervals as previously.

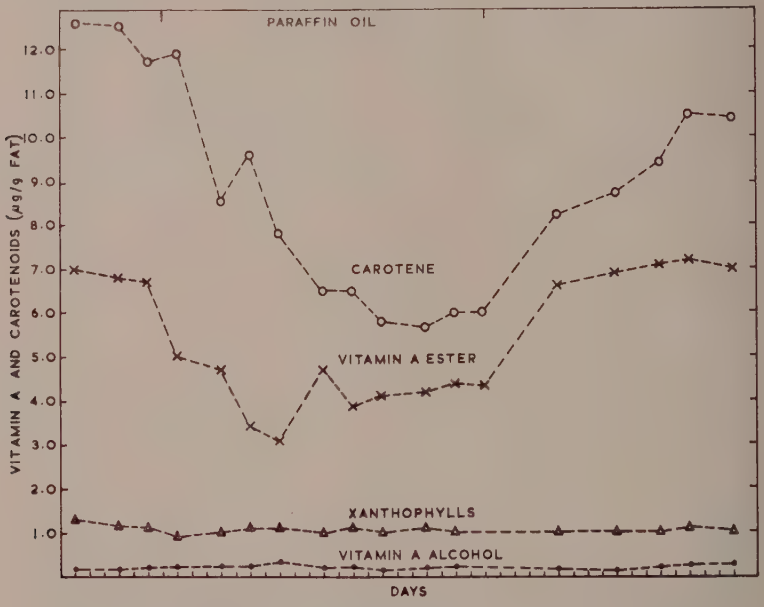

Fig. 2.—Effect of 300 ml. paraffin oil daily on the levels of carotenoids and vitamin A in the butterfat of cows. Carotene o — o; xanthophylls Δ — Δ; vitamin A ester x — x; vitamin A alcohol . — .

During all these experiments, milk samples were collected from untreated control cows. The number of such animals varied from 2 to 6 and in some cases figures for the levels of carotene and vitamin A in the butterfat from these animals were made available from the records of The Dairy Research Institute (N.Z.) through the courtesy of Dr. F. H. McDowall.

Butterfats were prepared for assay essentially as described by McGillivray (1957a), and vitamin A and carotenoids were estimated in butterfats, blood samples, and subcutaneous fats using the method of Thompson *et al.* (1949).

Results

Levels of carotenoids and vitamin A in the blood plasma and butterfat of the two cows dosed with 300 ml. paraffin oil daily (Exp. I) are shown in Fig. 1 and 2. In the blood plasma, the administration of

oil produced a marked depression in carotene, the levels however returning rapidly to normal during the post-experimental period. Xanthophyll levels were also depressed to some extent relative to controls. Plasma vitamin A alcohol levels were unaffected by treatment. Vitamin A ester levels (not included in the Fig.) were too low for accurate measurement, but did appear to be depressed somewhat during the treatment period. As is normal at that time of the year (McGillivray, 1957a), levels of carotenoid in the blood of the control cows increased steadily throughout the experimental period.

In the butterfat, xanthophyll and vitamin A alcohol levels remained unchanged but there was a marked decrease in the levels of vitamin A ester and carotene, the depression in the latter closely paralleling that in the blood. Levels of vitamin A and the carotenoids in the butterfat of the control cows remained constant throughout the period and have therefore been omitted from Fig. 2.

Levels of carotenoids and vitamin A in the subcutaneous fat are shown in Table 1. During the period of paraffin treatment a slight increase occurred in all levels.

TABLE 1. Levels of Carotenoids and Vitamin A in Subcutaneous-fat Samples from Two Jersey Cows

	Carotenoids		Vitamin A	
	Carotene	Xanthophylls	Alcohol	Ester
	μg/g fat			
Pre-experimental				
Cow 1	5.4	0.8	0.3	0.6
Cow 2	6.7	1.0	0.4	0.7
Average	6.1	0.9	0.4	0.7
Paraffin dosed 20 days				
Cow 1	7.8	0.6	0.8	1.3
Cow 2	7.7	0.4	0.8	1.3
Average	7.8	0.5	0.8	1.3

Fig. 3 shows the levels of carotene in the blood plasma and of carotene and vitamin A ester in the butterfat of individual cows dosed with 30, 60, and 120 g. paraffin oil daily (Exp. II). The treatment had no effect on blood vitamin A alcohol or on butterfat vitamin A alcohol and xanthophyll levels, and these have been omitted from Fig. 3. Blood plasma vitamin A ester was again too low for accurate estimation but did appear to be depressed slightly by the oil. Throughout the experiment, levels of carotene and vitamin A again remained relatively constant in the butterfat of the control cows.

Fig. 4 shows the effect of saturating the paraffin oil with carotene (Exp. III). When one cow was dosed with 225 ml. paraffin oil daily, the normal depression in butterfat carotene and vitamin A ester was

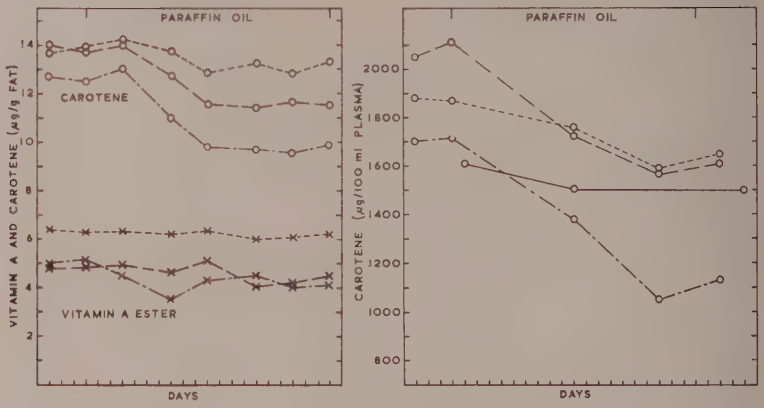

Fig. 3.—Effect of 30, 60, and 120 ml. paraffin oil daily on the levels of carotenoids and vitamin A in the butterfat and blood of cows. Control ———; 30 ml. – – –; 60 ml. — — —; 120 ml. — – —; Carotene o — o; vitamin A ester x — x.

obtained, but when a similar animal received the same amount of paraffin saturated with carotene the depression was considerably less. Similarly in the blood, the carotene level was depressed from 1,300 to 700 µg/100 ml. plasma on dosing with paraffin oil alone, but when the oil was saturated with carotene the depression was from 1,250 to 1,000 µg/100 ml.

The effect on butterfat carotenoids and vitamin A levels of administering a single dose of 2 g. vitamin A either alone or mixed with paraffin oil is shown in Table 2. Clearly paraffin oil has little effect on the utilization of preformed vitamin A.

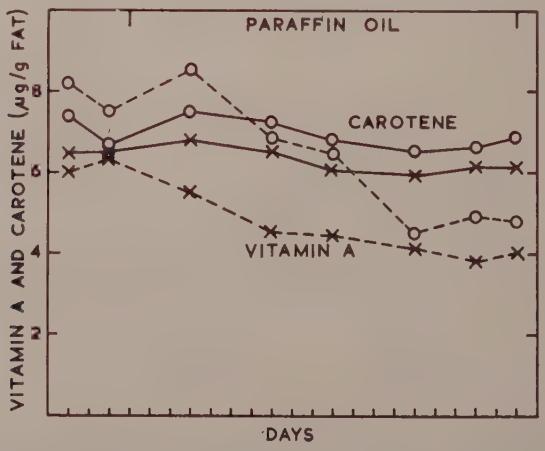

Fig. 4.—Effect of 225 ml. paraffin oil alone or saturated with carotene, on the levels of carotenoids and vitamin A in the butterfat of cows. Paraffin oil alone - - - -; paraffin oil saturated with carotene ———. Carotene o — o; vitamin A ester x — x.

TABLE 2. Levels of Carotenoids and Vitamin A in the Butterfat of two Cows, One Dosed with 2 g. Vitamin A (Control) and the Other Dosed with the Same Quantity of Vitamin A dissolved in 225 ml. Paraffin Oil (Treated)

Days after Dosing	Carotenoids				Vitamin A			
	Carotene		Xanthophylls		Alcohol		Ester	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Pre-experimental average	4.6	9.1	0.5	3.9	0.29	0.30	5.3	6.2
1	4.9	8.2	0.3	3.2	2.2	1.9	54	54
2	5.3	8.5	0.3	3.0	0.61	0.82	32	27
3	5.1	8.2	0.4	3.4	0.35	0.53	15	12
4	4.8	8.0	0.4	3.5	0.30	0.35	7.8	8.3

Discussion

From the results presented here it is apparent that paraffin oil, even at relatively low dosage levels, produces a depression in the carotene content of the blood plasma and butterfat of cows. Vitamin A ester in the milk fat, and probably in the blood plasma, is also depressed, but the oil has no effect on the blood plasma or butterfat levels of vitamin A alcohol.

The effect of mineral oils on the utilization of carotene by humans and by experimental animals has been investigated by a number of workers (McDowall *et al.*, 1957). It appears that the carotene is partitioned between the digestible fats in the feed and the non-digestible paraffin oil so that, in effect, less carotene is available for absorption and conversion to vitamin A. The extent of the decrease in this "available" carotene depends on the relative amounts of digestible fat and paraffin oil and on the relative solubilities of carotene in the fat and the paraffin. Thus Curtis and Ballmer (1939) found that paraffin oil, previously saturated with carotene, had no effect on carotene utilization, and in the present investigation the use of a similar solution produced relatively little change in butterfat or blood-plasma levels. Assuming that there is a similar partition of pasture carotene between the pasture fat and the paraffin oil, the changes in blood plasma and butterfat carotene and vitamin A levels may be readily explained.

In cattle, a portion of the absorbed carotene is converted to vitamin A in the wall of the intestine (Kon *et al.*, 1955). This vitamin A, as the ester, together with unchanged carotene, is associated, during transport from the intestines to the storage organs, with dietary fat in the chylomicrons, the uptake of which by the mammary gland provides the main source of butterfat vitamin A and carotene (McGillivray, 1957b). Any decrease in carotene absorption would therefore cause, almost immediately, a corresponding decrease in butterfat carotene. This relationship between paraffin intake and depression of carotene content of the butterfat is shown in Fig. 5. The data for this figure

are taken from Figs. 2, 3, and 4, and, for the daily intake of 150 ml. oil, from the previous communication (McDowall *et al.*, 1957). Despite the differences in the method of administration of the oil and in the stage of lactation of the animals, a fairly linear relationship is apparent. No quantitative information is available regarding the effect of reduced carotene absorption on the proportion of carotene which is converted to vitamin A in the intestine. However from the fairly linear relationship between the depression in butterfat vitamin A ester and paraffin intake (Fig. 5) it would appear that a reduction in carotene intake is associated with a corresponding but slightly less pronounced reduction in vitamin A formation. This is supported by the apparent slight decrease in blood-plasma vitamin A ester levels during periods of paraffin dosing.

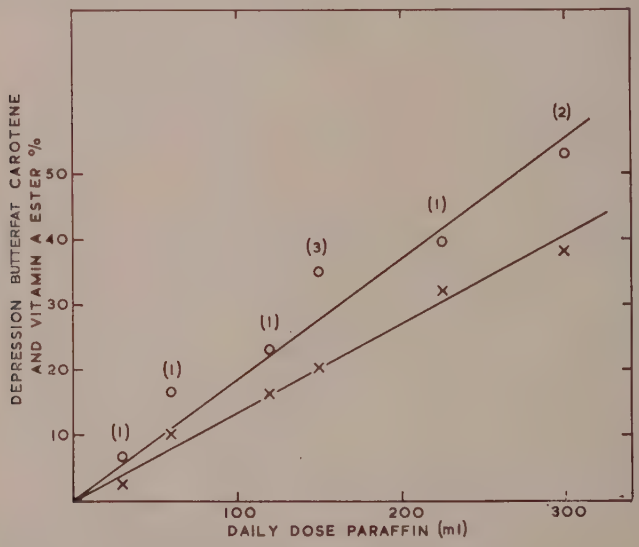

Fig. 5.—Relationship between dosage level of paraffin oil and depression of butterfat carotene and vitamin A ester. Figures in brackets show number of cows at each level. Carotene o — o; vitamin A ester x — x.

In the blood plasma, the level of carotene at any time represents an equilibrium between the rate of removal from the blood, either through destruction or withdrawal by the tissues, and the rate of uptake of unchanged carotene through the intestine. Thus any decrease in absorption of carotene would result in a corresponding decrease in blood-plasma carotene levels. No change in blood vitamin A alcohol would be expected since these levels are maintained from liver reserves and are virtually independent of immediate carotene or vitamin A intakes.

From results such as those presented in Fig. 1, some information may be deduced regarding the rate of uptake of carotene from the intestine and the rate of its destruction or removal from the blood of cows on pasture. The animals used in Exp. I weighed approximately 500 kg. Assuming that, in the cow, blood constitutes 7.7% of the body weight (Dukes, 1947) and contains 50% of plasma, the plasma volume of these

animals was about 20 litres. Thus during the pre-experimental period the blood contained about 250 mg. carotene. This decreased to about 150 mg. during the first 8 days of paraffin administration, a daily loss of 12 mg. During the post-experimental period the carotene in the blood rose from about 130 mg. to 330 mg. over a period of 17 days, a daily increase of about 12 mg. Since steady levels of carotene are attained in the blood following changes in carotene intake it seems reasonable to assume that the rate of removal or destruction of carotene from the blood is proportional to concentration. The equilibrium level of carotene in the blood will therefore be proportional to carotene intake and from the blood levels in this experiment it follows that dosing with paraffin oil reduced the carotene intake to approximately 650/1,700 or 38% normal. The difference between the mean rate of destruction over the 8 days and this reduced intake was equal to 12 mg/day, whence it follows that normal carotene absorption, over and above that converted to vitamin A during absorption, must have been of the order of 40–50 mg. per day. To this must be added the 4–5 mg. carotene secreted daily in the milk. Although the total seems high, it represents only about 1% of the daily intake of carotene from pasture. Most of the carotene absorbed unchanged through the intestines must be destroyed, probably in the blood, otherwise much higher levels would be found in bovine storage tissues, particularly the body fat. The levels of carotene in the subcutaneous fat (Table 1) were relatively low (of the same order as the butterfat) and there was no evidence of any release of carotene from the fat to maintain blood levels during the period of reduced carotene intake.

If it is correct to assume that the bulk of the carotene and vitamin A in butterfat is of immediate dietary origin (McGillivray, 1957b), then it follows that the ratio of vitamin A to carotene in the butterfat gives some indication of the relative quantities of these materials leaving the intestinal wall during periods of normal carotene absorption. During the pre- and post-experimental periods in Exp. I therefore, the daily intake of vitamin A must have been of the order of 25 mg.

From the blood and butterfat levels shown in Fig. 1 and 2 it is clear that the paraffin oil had little effect on the absorption of xanthophylls. Being more polar than carotene, it is probable that these would be less soluble in the paraffin oil and thus their absorption less influenced by it. This is in agreement with the finding that the absorption of preformed vitamin A alcohol (again more polar than carotene) was not affected to any extent by the simultaneous administration of paraffin oil.

Conclusions

From the results presented in this and the preceding paper (McDowall *et al.*, 1957) it is apparent that oral dosing with paraffin oil, at levels found effective for the control of bloat, will produce a marked reduction in the carotene content of butterfat. This is accompanied by some reduction in the level of vitamin A ester and probably of other fat-soluble substances (depending on their relative solubilities in paraffin oil and pasture fats). While some reduction in the colour of our butter may be desirable economically, the importance of milk and its products as sources of vitamin A in the human diet cannot be overstressed (e.g. McGillivray and Thompson, 1957). Serious consideration

must therefore be given to nutritional aspects of the problem. It should be borne in mind, however, that the vitamin A and carotene would be reduced for a short time only and that during the spring months cows dosed with 150 ml. paraffin oil per day would produce butterfat with a total vitamin A potency of the order of 40 I.U. /g., a figure which is still higher than our normal mid-summer level. Furthermore, since the absorption of pre-formed vitamin A does not appear to be impaired by the paraffin, the reduced total vitamin A potency of the butterfat could be readily compensated by the inclusion, at a very little cost, of a small quantity of a vitamin A concentrate in the paraffin oil.

As far as the wellbeing of the cows themselves is concerned, in view of the high intake of all fat-soluble vitamins from the pasture (and presumably therefore high tissue-stores of these substances) it appears unlikely that the use of reasonable levels of paraffin oil for relatively short periods would have any adverse effects. There may well be, however, other aspects of the effect of paraffin oil on the animals, which require further investigation.

Acknowledgements

The author is grateful to the Department of Scientific and Industrial Research for a grant towards the investigation; also to Mr. B. W. Taylor and the farm staff of The Dairy Research Institute (N.Z.) for assistance with the dosing of the animals and the collection of milk samples; and to Mr. C. S. W. Reid, Grasslands Division, Department of Scientific and Industrial Research for taking the subcutaneous fat samples. The assistance of Miss Fay Frecklington with some of the experimental work is also acknowledged.

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CORRIGENDUM

Volume 38, June, page 779, eighth line from bottom: *for* **Nerine curvifolia** Herb. var. **fothergillii** Baker *read* **Nicotiana tabacum** L.

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